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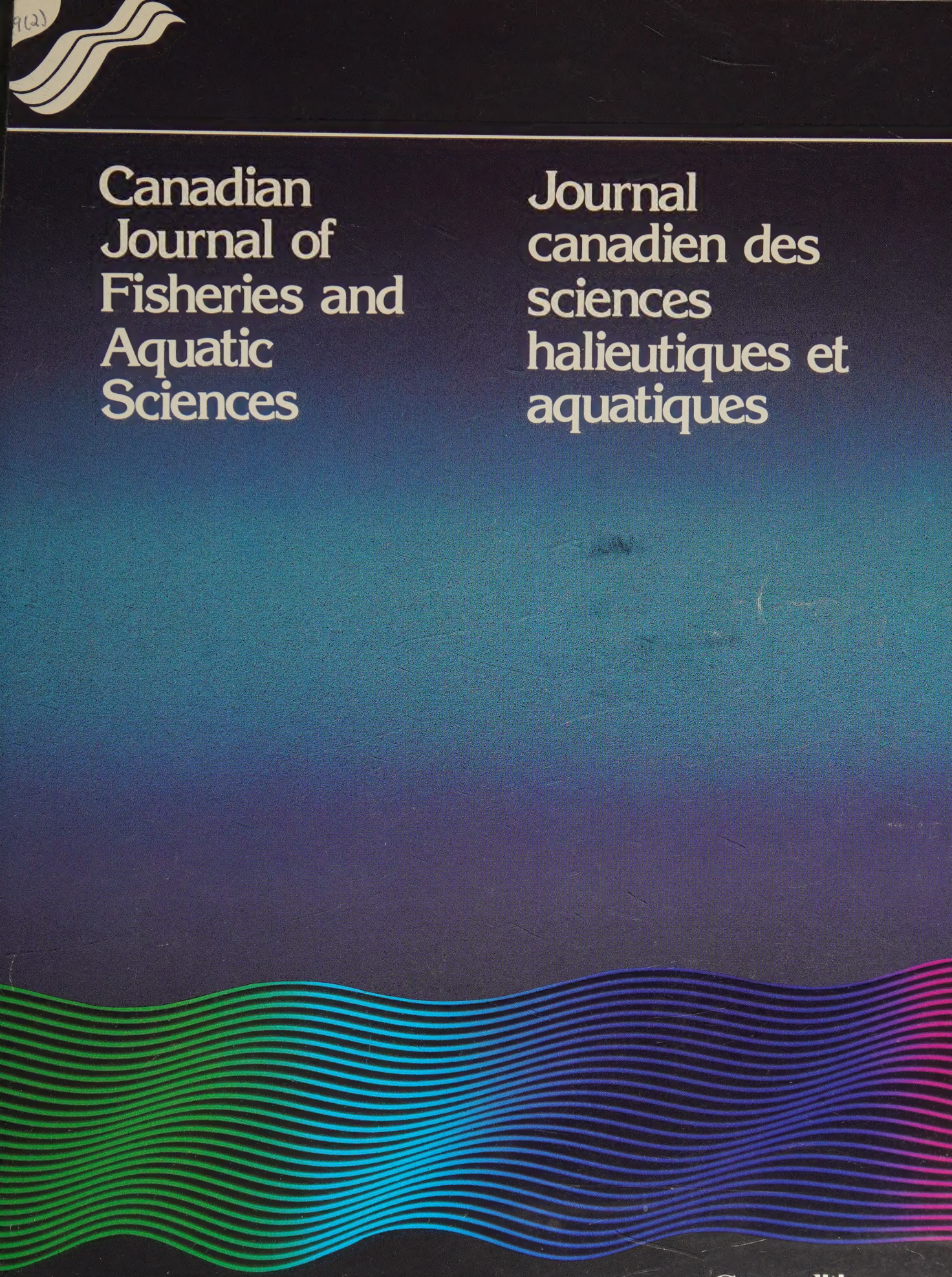

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Indices of Total Stock Biomass in the "Northern" and Gulf of St. Lawrence Atlantic Cod (*Gadus morhua*) Stocks Derived from Time Series Analyses of Fixed Gear (Trap) Catches

G. A. Rose

Department of Fisheries and Oceans, Science Branch, St. John's, Nfld. A1C 5X1, Canada

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The hypothesis that annual catches of fixed gear fisheries are cross-correlated with stock biomass at lags predictable on the basis of the relative ages of fish comprising the catch and biomass was verified for the trapnet fisheries of the northeastern Newfoundland "northern" (NAFO 2J3KL) and northern "Gulf" of St. Lawrence (NAFO 3Pn4RS) Atlantic cod (*Gadus morhua*) stocks. Time series indices of stock biomass were derived from these cross-correlations. For northern cod, the index was a 3-yr weighted and lagged moving average of catch. For the years 1972–81 (the first half of the available data) the trap index (T_i) was regressed on the stock biomass (B_i) determined by sequential population models (SPA) ($T_i = 0.477B_i^{0.638}$, $r = 0.99$, $P < 0.01$). Biomass forecasts for 1982–90 derived from this function (inverted) were positively correlated with recent SPA-based estimates ($r = 0.94$, $P < 0.02$). For Gulf cod, the index was a 4-yr weighted and lagged moving average of catch. This index was regressed on SPA-determined biomass for the years 1974–81 ($T_i = -3.19 + 0.0217B_i$, $r = 0.99$; $P < 0.01$). Biomass forecasts for 1982–90 were positively correlated with (but lower than) SPA-based biomass estimates for the Gulf stock ($r = 0.91$, $P < 0.05$).

L'auteur a vérifié l'hypothèse selon laquelle les prises annuelles de morue franche (*Gadus morhua*) réalisées à l'aide de parcs en filet dans les sous-zones 2J3KL et 3Pn4RS de l'OPANO sont en corrélation croisée avec la biomasse du stock à des décalages prévisibles en fonction des âges relatifs des poissons capturés et de la biomasse. Les indices de la série temporelle de la biomasse du stock ont été dérivés à l'aide de ces corrélations croisées. Dans le cas de la morue franche, la moyenne mobile des prises pondérée et retardée s'élevait à 3 ans. Pour les années entre 1972 et 1981 (soit la première moitié des données disponibles), on a effectué une régression de l'indice concernant les trappes (T_i) en fonction de la biomasse du stock (B_i) déterminée à l'aide d'une analyse de population séquentielle (APS) ($T_i = 0{,}477B_i^{0{,}638}$, $r = 0{,}99$, $P < 0{,}01$). Les prévisions de la biomasse pour la période 1982–1990 dérivées à l'aide de cette fonction (inversée) étaient en corrélation positive avec de récentes estimations basées sur des APS. Dans le présent cas, la moyenne mobile des prises pondérée et retardée s'élevait à 4 ans. On a ensuite effectué une régression de cet indice en fonction de la biomasse déterminée par APS pour les années 1974–1981 ($T_i = -3{,}19 + 0{,}0217B_i$, $r = 0{,}99$, $P < 0{,}01$). Les prévisions de la biomasse pour la période 1982–1990 étaient en corrélation positive avec (mais moins élevées que) les estimations de la biomasse basées sur des APS du stock de morue franche du golfe du Saint-Laurent ($r = 0{,}91$, $P < 0{,}05$).

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Empirical indices of fish abundance underpin most fisheries assessments. These indices are typically employed as predictors of the state of stocks and as independent "calibrators" for more complex population models (Gulland 1965; Pope 1972). In marine systems, abundance indices have often been derived from the catch rates of mobile fishing fleets (Gavaris 1980; Shepherd 1988). However, in many cases these indices have proven to be unreliable because variability in catchability (q) and fishing power have led to unpredictable trends or short-term variations (or both) in fishing mortality (Paloheimo and Dickie 1964; Rothschild 1977). Hence, fish abundance is often only poorly correlated with the catch rates of mobile fleets (e.g. Rose and Leggett 1989a, 1991).

Abundance indices can also be derived from the catches of immobile fixed gear (traps, pounds, fyke nets). Fixed gear indices have been used extensively in North American freshwater systems, especially to monitor the abundance of migrating fish (Hamley and Howley 1985). In general, this usage has not been applied to marine systems, despite evidence showing that indices based on fixed gear catches are significantly correlated with independent estimates of fish abundance in both freshwater (e.g. Forney 1961; Yeh 1977; Ryan 1984) and marine systems (Pinhorn 1986; Miller and Nakashima 1987; Rose and Leggett 1988, 1989a).

The development and application of abundance indices derived from the catches of commercial fixed gear fisheries have been constrained by (1) the lack of controlled sampling designs, (2) undocumented effort levels, and (3) gear selectivity (species, size, age) (Hamley and Howley 1985). However, in many traditional fixed gear fisheries, variability in sampling and real effort (sensu Rothschild 1977) may be mollified because the good locations for gear, those combining suitable bathymetry with high potential to intercept migrating fish, tend to be limited in number, well known, and in use seasonally on

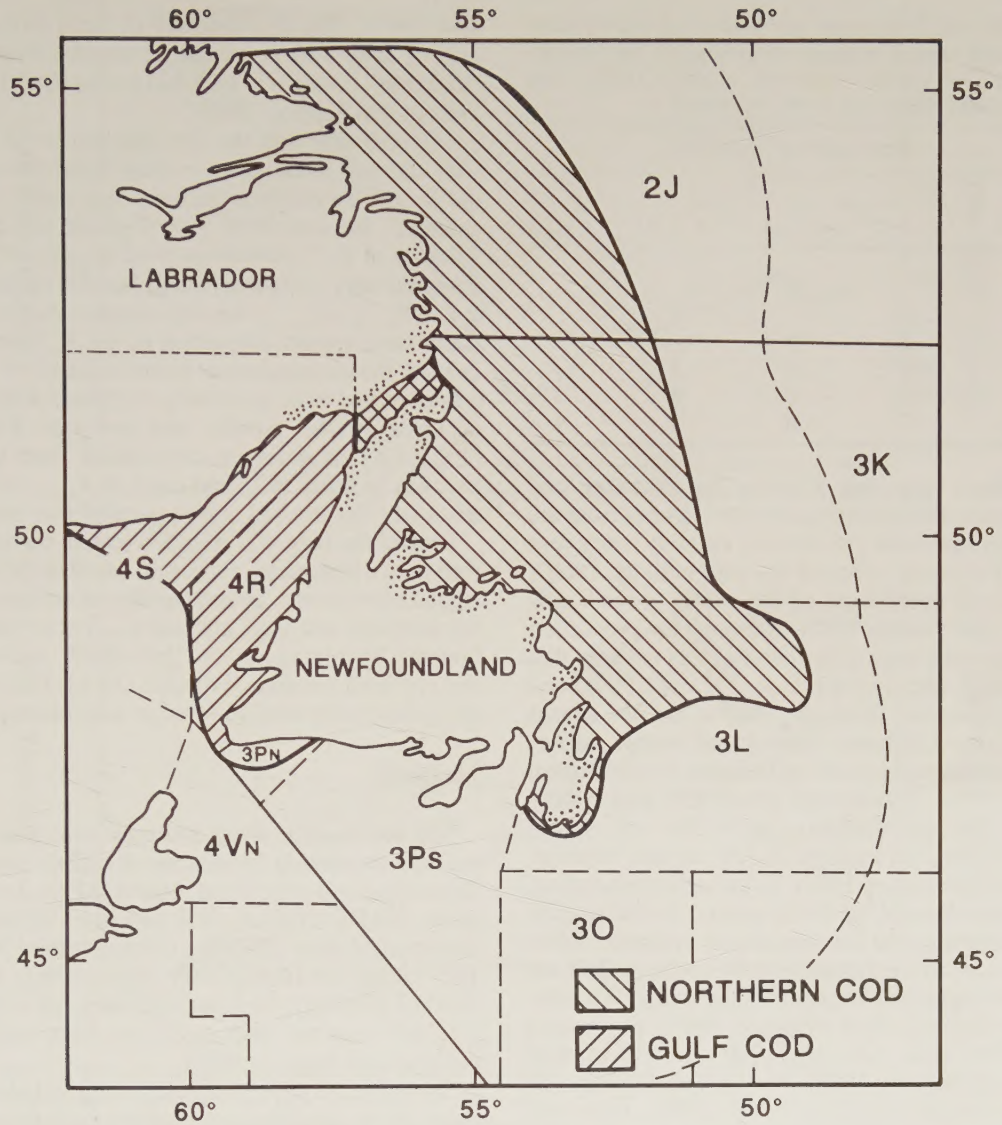

FIG. 1. Geographical range of the "northern" (NAFO 2J3KL) and northern "Gulf" of St. Lawrence (NAFO 3Pn4RS) cod stocks. Note the range overlap of these two stocks in the vicinity of the Strait of Belle Isle. The distributions of the trap fisheries for these stocks are indicated by hatching. The broken line outlines a portion of Canada's 200-mile limit.

a regular basis. The deployment of additional gear at less optimal sites or times may have little impact on real effort in these fisheries. Moreover, variability in sampling and effort is likely to be random in nature because effort tends to be expended relative to fish availability inshore, which itself responds to random environmental and biological variations (Rose and Leggett 1988, 1989a). Furthermore, many fixed gear fisheries have been prosecuted in a consistent manner, with little evidence of increasing power, and without the constraint of catch quotas, for many years (see von Brandt 1972; Whiteley 1977). As a consequence, at typical fishing levels that do not saturate capacities, the stationarity of real effort and sampling in fixed gear fisheries may be much greater than in mobile gear fisheries.

The selectivity characteristics of a fishing gear, relative to the age structure of the fished population, to a large extent determine the temporal relationships between catch and abundance. For example, if on the one hand a gear selects for younger fish from a population whose biomass is dominated by fish of at least this age, then catch levels in specific previous years would be expected to be correlated most strongly with present overall population biomass. On the other hand, catches by gear that selects for older fish from the same population would be less likely to be correlated with total biomass at any time.

In this paper I test the hypothesis that in fisheries which use gear that selects for younger fish (typical of some trap fisheries) the strengths and signs of the lagged correlations between catch and biomass are predictable and thus could be used to derive a time series based forecasting index of stock biomass. This analysis uses data from the Atlantic cod (*Gadus morhua*) trap fisheries in Atlantic Canada.

Atlantic Cod Trap Fisheries

The trap fisheries for Atlantic cod on the Canadian east coast have been prosecuted for nearly a century (Whiteley 1977). The two largest stocks in this region, the "northern" cod (NAFO 2J3KL) and the northern "Gulf" cod (NAFO 3Pn4RS), have

TABLE 1. Ages of fish which dominate any year's (y_n) total fishable biomass (>3 yr of age) relative to those comprising the ages caught in the trap fisheries (3–7 yr) for "northern" (NAFO 2J3KL) and "Gulf" (NAFO 3Pn4RS) cod stocks in the previous 5 yr.

Ages of fishable biomass in year n	Ages caught in trap fishery				
	$n-1$	$n-2$	$n-3$	$n-4$	$n-5$
3					
4	3				
5	4	3			
6	5	4	3		
7	6	5	4	3	
8	7	6	5	4	3
9		7	6	5	4

traditionally sustained large trap fisheries for migrating cod (Fig. 1). For example, the total annual inshore catches from the northern cod fishery exceeded 175 000 t in virtually every year from the late 1800's (the inception of the trap) until the 1960's and the occurrence of overfishing on the winter grounds offshore (Lear et al. 1986; Harris 1990). Although the proportion of those catches taken by traps is not certain, it is unlikely that it was less than 40% (the average from 1975 to 1989), and probably was much higher. Since the 1960's, and the advent of gillnet and otter trawl fisheries, inshore and trapnet catches declined both in absolute magnitude and relative to overall total catches by all gear types. For example, since 1959, trap catches have averaged 37 000 t per annum (range 14 000 – 64 000) in the northern cod fishery, an average of 12% of total landings (range 4–27) (Baird and Bishop 1989). Trap catches of northern cod have never been limited by catch quotas. In the smaller Gulf trap fishery, historic total landings are not available. However, historic accounts of the fisheries in the northern Gulf are suggestive of much higher landings than occur today (Whiteley 1977). Total trap landings have averaged 4000 t per annum (range 1000–8000 t) since the early 1960's (2–9% of total catches) (Fréchet and Schwab 1989). The Gulf trap fishery has been subject to an inshore allocation in the 1980's. However, allocations appear to have had little effect on catches. Catches have declined during the 1980's, from levels well above the allocation to levels well below it (Fréchet and Schwab 1989). Trap catches are typically highly variable from year to year in both the northern and Gulf cod fisheries (Lear et al. 1986; Rose and Leggett 1988).

The methods of the cod trap fishery have changed little since their inception at Bonne Esperance on the north shore of the Gulf of St. Lawrence in 1871. Trap berths are typically located in shallow waters (<20 m, some deeper) on grounds known to be travelled by cod in search of their capelin (*Mallotus villosus*) prey. Trap grounds are widespread over most of the coastline of Newfoundland and Labrador adjacent to the range of the northern cod, less so for Gulf cod (Fig. 1). Many trap "berths" have been occupied by fishing families for several generations (at present in Newfoundland, berths are allocated by an annual draw). The best berths are limited in number, locally well known, and, in general, fished consistently. For example, the original berths at Bonne Esperance were still in use in 1986, 115 yr after they were first occupied.

The cod trap was designed to catch relatively young migratory cod (Whiteley 1977). Trap catches of cod from both the northern and Gulf stocks are typically dominated by young fish, of ages 3–6 or 7 yr (Baird and Bishop 1989; Fréchet and Chouinard 1987). The age structure of these two stocks is similar. Fish of ages <10 yr typically comprise over 95% of the total "fishable" biomass in both cod stocks (Baird and Bishop 1989; Fréchet and Schwab 1989).

I hypothesize that the age selectivities of the northern and Gulf cod trap fisheries are evident in the strengths and signs of the lagged correlations between trap catch and biomass. For example, the cohorts of 3- to 7-yr-old fish caught in the trap fisheries of Y_{n-5} would be 8–12 yr old in Y_n and expected to comprise only a relatively small fraction of the overall biomass. But in Y_{n-4} to Y_{n-1}, the trap-caught cohorts would on average comprise a greater proportion of the Y_n biomass. As a consequence, the average annual biomass (ages >3 yr) of both stocks was expected to be positively correlated with trap catches that occurred in the present and previous 4 yr (lags 0–4 yr) (Table 1). The strongest correlations were expected to occur between biomass in Y_n and catch in Y_{n-1} to Y_{n-3} because at these lags the level of cohort overlap was maximal.

I tested the lagged correlation (catch–biomass) hypothesis. I then used a time series approach, based on the strengths of these lagged correlations, to derive relative indices of the biomass of the northern and Gulf cod stocks. These indices were "calibrated" by regression on "absolute" measures of biomass derived from population models (SPA). Following calibration, the indices were used to forecast total biomass in these stocks.

Methods

The data used in these analyses were total trap catches and average population biomasses of cod of ages >3 yr derived from sequential population models (SPA). For the northern cod stock (NAFO 2J3KL), SPA estimates of biomass have been documented since 1962; trap catch data have been collated since 1969 (Baird and Bishop 1989; Harris 1990). For the Gulf stock (NAFO 3Pn4RS), biomass estimates from SPA are available for 1974 onward; trap catch has been collated since 1965 (Fréchet and Schwab 1989).

To derive the trapnet-based abundance indices, the trap catch series were smoothed to reduce high-frequency variability believed to relate primarily to environmental factors and influences not related to stock abundance (Lear et al. 1986; Rose and Leggett 1988, 1989a). To reflect the lagged structure of the correlation between trap catch and biomass (see Fig. 3), each year's index is a weighted average of the total trap catches in specific earlier years. The weights were the normalized r^2 of cross-correlations having $P < 0.05$. The smoothing filter was thus a lagged and weighted moving average:

$$(1) \quad T = \sum_{i=-1}^{l} (r_i^2 \cdot c_i)$$

where T = the abundance index, l = the number of lags of the full series of biomass and trap catch (catch leading) having correlations with $P < 0.05$, r_i^2 = the normalized ($\Sigma r_i^2 = 1$) squared correlation coefficients at each lag, and c_i = trap catch for each year (i).

To develop forecasting tools, I used the approach recommended by Sissenwine et al. (1983), that is, "to use historic VPA or cohort analysis estimates to calibrate. . .estimates and to then use calibrated. . .results to estimate population size in the most recent years." The trap-based abundance indices were "calibrated" using the "inversion" procedure. This method minimizes the mean square error of the predictions in cases

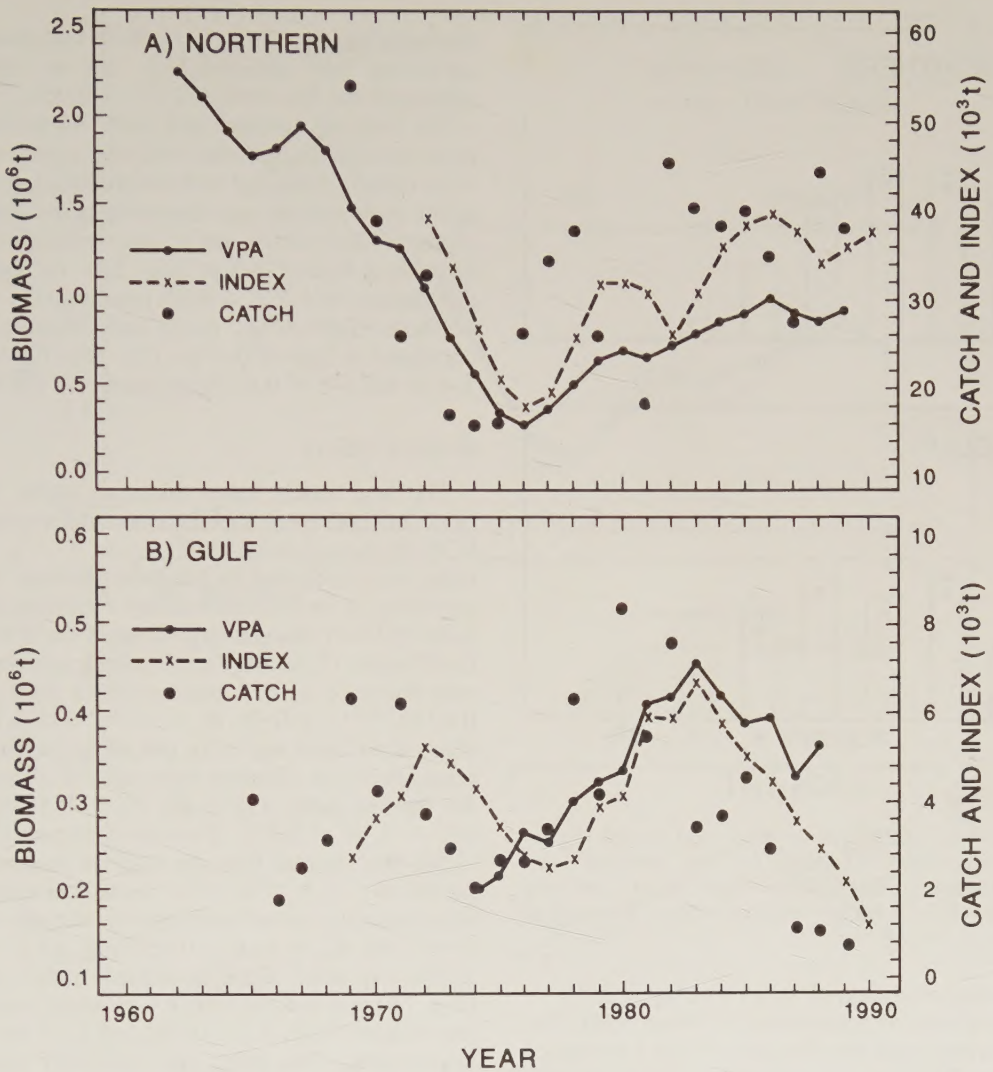

FIG. 2. SPA-determined biomass, total annual trap catch, and the calculated trap index for (A) the "northern" cod stock (NAFO 2J3KL) and (B) the northern "Gulf" of St. Lawrence cod stock (NAFO 3Pn4RS). See text for details of the calculation of the indices.

where both the dependent and independent variables contain error (Lee and Yum 1989). The abundance indices derived from trap catches were regressed on the SPA biomass estimates for the earlier years (1972–81 for northern cod; 1974–82 for Gulf cod). These earlier SPA estimates (the converged parts of the series) were the most reliable because all cohorts had passed fully through the fishery (Winters 1988).

An empirical approach was employed in model development: inspection of the data indicated that a linear model would be appropriate for the Gulf stock but that a multiplicative model would provide a better fit to the wider ranging and nonlinear data from the northern cod stock. These models were inverted to forecast biomass in more recent years. Forecasts were compared with recent SPA estimates of biomass for the same period. SPA estimates are considered to have been derived independently of the trap forecasts (although trap catch comprised up to 20% of the annual total catch statistics upon which the SPA is based) because trap catches were only poorly correlated with the overall total catch of all gear types (including traps) in both the northern ($r = 0.36$, $df_e = 11$, $P > 0.10$) and Gulf stocks ($r = 0.55$, $df_e = 10$, $P = 0.06$). Moreover, the SPA's were themselves calibrated with research vessel and offshore catch rate indices derived independently from the trap fisheries.

Statistical analyses and model development employed cross-correlation and regression techniques (Fuller 1976). In keeping with Warren (1986), statistical results and interpretations are divorced (probability levels rather than the word "significant" are given for all comparisons). All index and biomass series showed evidence of autocorrelation, and thus lack of independence, between successive years. Therefore, the probability level of each correlation was assessed in terms of the effective number of independent observations, n_e, rather than the full n, according to

$$(2) \quad n_e = n^{-1} + 2n^{-2} \sum_{j=1}^{n-1} (n-j)p(jt)$$

where $p(jt)$ is the autocorrelation at lag jt and t is the sampling interval (Bayley and Hammersley 1946; Garrett and Petrie 1981). Autocorrelations having $P > 0.05$ were excluded. The SE of the cross-correlation coefficients was determined as

$$(3) \quad \text{SE}\, r_{k1k2}(h) = [n\text{-}1 \sum_{p=-s}^{s} r_{k1k1}(p)\, r_{k2k2}(p)]^{0.5}$$

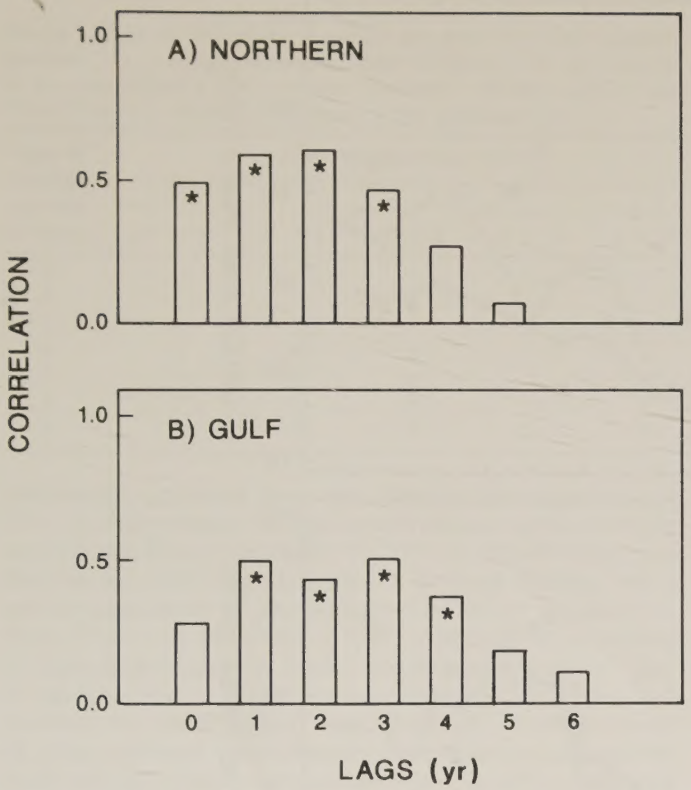

FIG. 3. Cross-correlation coefficients between total annual trapnet catch and stock biomass (ages >3 yr) for (A) the "northern" cod stock (NAFO 2J3KL) and (B) the northern "Gulf" of St. Lawrence cod stock (NAFO 3Pn4RS). A lag of 1 indicates catch 1 yr ahead of the biomass estimate. $P < 0.05$.

where r_{kp} = the autocorrelation of series k at lag p and s = the lag where autocorrelation approaches 0 (Fuller 1976). The hypothesis of zero correlation was then tested with a two-tailed t-test (df_e based on n_e). Residuals were regressed against predicted values to test for trend (Framsted et al. 1985).

Results

SPA Biomass and Trap Catch

The biomass (as estimated by SPA) of the northern cod stock declined rapidly during the 1960's and until 1976 (Fig. 2A). From 1976 until the mid-1980's, there was a relatively steady increase in biomass. Since 1986, there has been no evidence of further increase in biomass. Total catches in the trap fishery also declined from the late 1960's until 1974. From 1975 until the mid-1980's, there was a trend toward higher catches. However, more recently, from the mid-1980's until 1990, no strong trend in total trap catches was evident.

As expected, high-frequency variability in total trap catch (1969–89) was common. For example, the poor 1981 catch of approximately 18 000 t was followed by a 1982 catch of almost 46 000 t. Catch variability at periods of less than a decade was high (Fig. 2A). Although there was evidence of 3- to 6-yr cycles in the latter part of the total catch series, no autocorrelation was evident (all P's > 0.05) in the full series.

The biomass of the northern Gulf cod stock also increased from 1974 to 1983, but has since shown a steady decline (Fig. 2B). Over the same period, total trap catch exhibited a similar overall pattern, increasing from 1974 until 1980 then declining rapidly. As in the northern cod stock, high-frequency variability was commonplace, but no autocorrelation was evident in the full series (all P's > 0.05).

For both the northern and Gulf cod stocks, the patterns of cross-correlation between total trap catch and stock biomass were virtually identical to those predicted, given the selectivity of the traps and the age structures of the populations. For the northern cod stock, catch was positively correlated with biomass at lags of 0–5 yr (Fig. 3A). At lags of 0–3 yr, these correlations had P's < 0.05 (maximum $r = 0.61$). For the northern Gulf stock, catch and biomass were positively correlated at lags of 0–6 yr (Fig. 3B). Correlations at lags of 1–4 yr had P's < 0.05 (maximum $r = 0.51$).

Biomass Indices

The trap catch based biomass index for northern cod (Fig. 2A) consisted of a lagged moving average having weights 0.36, 0.38, and 0.26 for Y_{n-1} to Y_{n-3}, respectively. The index was calibrated to absolute biomass values (tonnes) by regressing it on SPA-determined estimates of biomass for the years 1972–81 (the converged part of the SPA series). The trap-based index (T_i in 1000's of tonnes) was positively correlated with this series of SPA biomass (B_i in 1000's of tonnes) ($T_i = 0.477B_i^{0.638}$, $r = 0.99$, $df_e = 6$, $P < 0.01$). The residual errors showed no trend and were not autocorrelated. The slope and intercept of this equation were close to identical to those fitted for the full series (1972–89: $T_i = 0.515B_i^{0.623}$, $r = 0.96$, $df_e = 8$, $P < 0.01$). Biomass forecasts for the later years (1982–90), derived from the inverted equation from the earlier period ($B_i = 3.191T_i^{1.568}$), were positively correlated with independently derived estimates of biomass from the 1989 SPA ($r = 0.94$, $df_e = 4$, $P < 0.02$) (Fig. 4A).

The trap catch based abundance index for the Gulf stock (Fig. 2B) was derived from a 4-yr lagged moving average having weights 0.30, 0.23, 0.30, and 0.17 for Y_{n-1} to Y_{n-4}, respectively. This index was positively correlated with estimates of biomass in the Gulf stock derived from SPA for the years 1974–82. The best fit was linear ($T_i = -3.19 + 0.0217B_i$, $r = 0.99$, $df_e = 4$, $P < 0.05$). Residual errors showed neither trend nor significant autocorrelation. The parameters of the equation fitted from the earlier data were virtually identical to those fitted for the full series (1974–88: $T_i = -3.17 + 0.0214B_i$, $r = 0.92$, $df_e = 6$, $P < 0.05$). Forecasts of biomass for the period 1983–89, based on the inverted equation fitted for the earlier period ($B_i = 146.9 + 46.1T_i$), were positively correlated with SPA biomass estimates for the same period ($r = 0.91$, $df_e = 4$, $P < 0.05$) (Fig. 4B). However, the trap index predictions indicated a steeper decline in the Gulf stock in recent years than shown by the 1989 SPA.

Discussion

These analyses verified the hypothesis that the age compositions of fixed gear catches, relative to those of the parent stock, would predict the patterns of lagged correlation between total fixed gear catches and biomass (in the Atlantic cod trap fisheries). This result confirms earlier conclusions that fixed gear catch rates (in this case catch) can reflect average fish abundance at relatively small spatial and temporal scales (Rose and Leggett 1988, 1989a), but extends these conclusions to the very large spatial scales of whole stocks and temporal scales of decades.

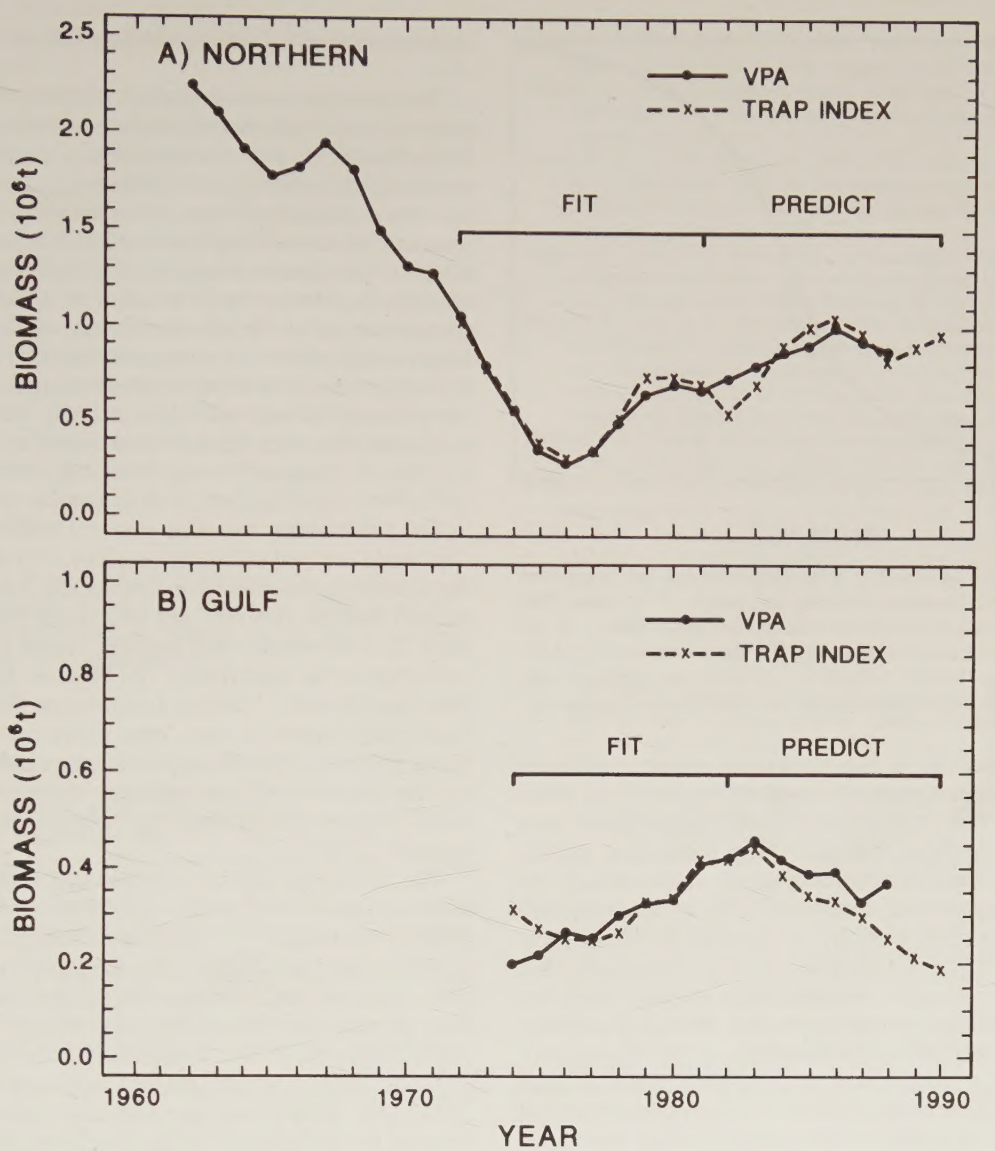

FIG. 4. Total stock biomass (ages >3 yr) as estimated by SPA (B_i in 1000's of tonnes) and from the trapnet indices (T_i in 1000's of tonnes) for (A) the "northern" cod stock (NAFO 2J3KL) and (B) the northern "Gulf" of St. Lawrence cod stocks (NAFO 3Pn4RS). For northern cod, the trap index was fit for the years 1972–81 ($B_i = 3.19T_i^{1.57}$); biomass levels for 1982–90 are true forecasts derived from this model. For Gulf cod, the index fit for the years 1974–82 ($B_i = 152.4 + 46.1T_i$) was used to forecast biomass levels in the years 1983–90.

It was noteworthy that the forms of the relationship between total trap catches and biomass appeared to differ between the two stocks investigated. However, the difference may relate more to differing ranges of the data available than to any fundamental difference between these fisheries. For example, the observed range of biomass levels in the Gulf fishery was perhaps too small (<0.3 million t), and at too low levels, to detect any nonlinear relationship which may occur over the full (but unknown) historical range of biomass and catch. Moreover, biomass varied only by a factor of 2 in this stock from 1974 to 1982, the period for which the model was fit. In contrast, the northern cod biomass varied by a factor of 5 from 1969 to 1981 (the model fit period) and the overall range of biomasses observed was much greater (>2 million t). In the northern cod stock, the observed nonlinearity in the catch–biomass relationship is suggestive of a density-dependent relationship between stock biomass and the availability of cod to the inshore fisheries. It is plausible that at relatively low biomass levels, a lesser proportion of the total cod biomass may undertake the onshore feeding migration (upon which the trap fishery depends) than at higher biomass levels. In keeping with this interpretation and its dependence on the interaction between cod and capelin, migrating cod have been shown to distribute themselves in proportion to capelin densities while inshore (Rose and Leggett 1989b, 1990), and a negative relationship between immature capelin abundance offshore and cod availability inshore has been reported (Akenhead et al. 1982).

The models presented here cannot be expected to reflect the full potential range of the relationships between biomass and catch in these fisheries. The shape of the full relationship is not known. However, I suggest that it takes a sigmoidal form (Fig. 5). Under this hypothesis, trap catches would be low and

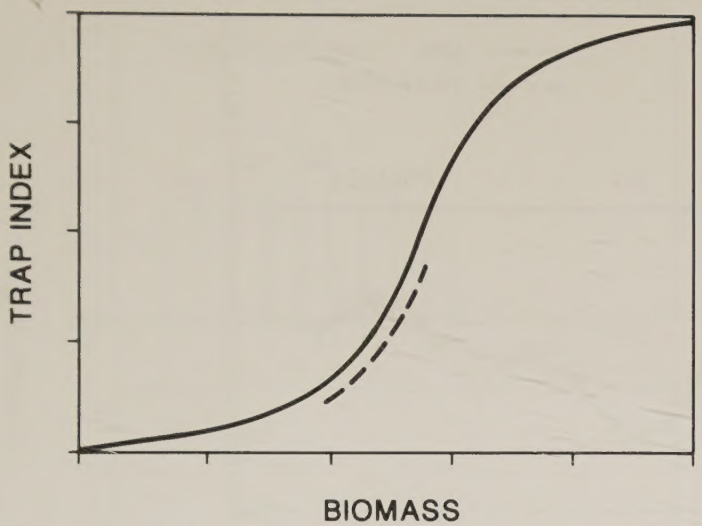

FIG. 5. Hypothesized sigmoidal relationship between the trap-based index and total stock biomass over the full range of biomass. The portions of the full model thought to correspond to the ranges of the observed values documented in this study for the "northern" (NAFO 2J3KL) and northern "Gulf" (3Pn4RS) cod stocks are shown by the broken line (range of Gulf values may be too small to show curvature).

unresponsive to changes in fish abundance at very low levels of biomass. At somewhat greater levels of biomass, real effort (the effect on the fish populations) would increase faster than biomass (as, on average, an increasing proportion of fish migrated onshore) and then remain relatively stable through the midranges of biomass. Here, catches show the greatest response to biomass and increase quickly. As biomass levels increase beyond the point of fishing saturation, total catches would reach an asymptote and real effort would steadily decline. Note that the present indices are expected to provide reliable predictions of stock biomass only within the midranges of the full range of biomass. Full-range models have not been parameterized from the data presented here because neither the lower nor upper end of the curve can be quantified with certainty; hence, the empirical approach was employed.

The empirical models developed here provide evidence that indices derived from fixed gear catches can provide reliable predictions of total stock biomass. The relationships demonstrated held reasonably well over a period of nearly two decades in two separate stocks. The data required as input for these time series models (in the case of the Atlantic Canada inshore fisheries) are readily available on an ongoing basis. I emphasize two precautions in the interpretation and potential use of these methods: (1) where full SPA methods are available, the time series method is intended to be complementary to those methods (to provide an additional and different view of fish stock status) and should not be considered as being competive with or replacement for more comprehensive population models and (2) the present techniques should be considered as a starting point for research and would likely be improved by better documentation of variations in nominal effort and environmental effects (Lear et al. 1986; Rose and Leggett 1988); these improvements in turn could lead to the development of annual age-structured trap-based abundance indices. Further studies will be required to assess whether or not these techniques can be used to "calibrate" SPA and its derivatives (Pope 1972), which require an independent index of abundance to estimate population size within several years of the most recent catch data.

The noted precautions notwithstanding, the accuracy of the predictions of these models relative to historic SPA-determined biomasses ($R^2 > 90\%$ for fitted series) suggests that variability in real effort, sampling, and environmental conditions, all unrelated to abundance changes, does not preclude the use of fixed gear catch data to derive biomass indices, as suggested by Lear et al. (1986). Rather, the variability attributable to factors unrelated to abundance can be reduced by a rational smoothing of the annual series. In this study, smoothing parameters were based on the observed correlation between catch and biomass over a period of years for which both catch and biomass levels have been relatively well documented. Any lower frequency variations in catches (these would appear as error trends caused by discrete changes in trap technology, fishing strategies, or real effort) could be dealt with in similar fashion.

The relationship between nominal effort (number of traps deployed) and real effort (the number of fish caught relative to the number in the stock) remains unclear. However, my results suggest that the two may not be closely related in these fisheries. It is noteworthy that reported recent declines in nominal trap effort in the Gulf fishery (A. Fréchet, Department of Fisheries and Oceans, Maurice Lamontagne Institute, Mont-Joli, Que., pers. comm.), have been correlated with stock abundance declines. The efficacy of the trap-based index, throughout this period of apparent changes in nominal effort, suggests that real effort is determined for the most part independently of nominal effort.

The robustness of the demonstrated relationships between inshore fishery catch indices and SPA-based estimates of the total biomass (ages >3 yr) in the cod stocks in NAFO 2J3KL (northern cod) and NAFO 3Pn4RS (Gulf cod), over two decades, suggests that inshore catches in both these fisheries have been directly and consistently responding to the abundance of fish at large cross- and alongshore scales during this period. This conclusion suggests that the stock complexes within NAFO 2J3KL and 3Pn4RS are geographical "units" and that variations in the abundances of any smaller scale stock units (e.g. inshore–offshore) have had negligible effects on the total catches of the inshore trap fisheries, at least during the past 20 yr.

It is illustrative to compare the recent biomass forecasts of the calibrated trap catch based indices with those of SPA models. Both methods suggest that the northern cod stock has grown slowly since the mid-1970's, but that biomass declined somewhat during the later period from 1986 to 1989. The trap index based predictions of biomass levels for the most recent years are consistent with those estimated by SPA (although they do suggest slightly lower levels of biomass for the most recent years) (Baird and Bishop 1989). For example, the trap-based index predicted northern cod biomass (age >3 yr) to be 0.80 million t in 1988 (95% CI = 0.5–1.4) and 0.92 million t in 1990 (95% CI = 0.6–1.5). The biomass predicted by SPA was 0.88 million t in 1988.

For the Gulf stock, both the calibrated trap index and SPA indicate a declining population. However, the trap index suggests a greater decline than indicated by the 1989 SPA. Specifically, the trap index predicted 1988 biomass levels to be 0.28 million t (95% CI = 0.2–0.4) and declining whereas SPA biomass estimates of the Gulf stock were approximately 0.37 million t (Fréchet and Schwab 1989). The present biomass of

Gulf cod can be projected by the trap-based index to be approximately 0.2 million t.

The time series averaging techniques demonstrated here for fixed gear fisheries may be useful in developing biomass indices applicable to traditional fisheries other than for Atlantic cod. The techniques could be modified for cases for which no independent estimates of historic biomass levels exist (lags and averaging weights for the smoothing filter could be estimated on the basis of the overlap of the age structures of catch and biomass). Used in this way, the technique could provide relative abundance indices where more direct and comprehensive population surveys and analyses are not feasible. The strength of these empirical approaches is believed to lie not only in the demonstrated accuracy and power of their predictions, but also in the potential availability of long time series of comparable catch data from traditional fixed gear fisheries.

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Fecundity and the Stock–Recruitment Relationship

J. Anthony Koslow

Division of Fisheries, CSIRO Marine Laboratories, G.P.O. Box 1538, Hobart 7001, Tasmania, Australia

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The stability of fishery population models often depends upon the form of the underlying stock–recruitment (SR) relationship, yet this relationship generally cannot be defined from actual fishery data. I use a simple SR model to show that the ability to characterize a SR relationship depends upon both natural mortality (M) during the prerecruit period, which is associated with a species' fecundity ($\mathcal{F}$), and the variability of M. The SR relationship may be readily determined in species with low fecundities (e.g. higher vertebrates: $\mathcal{F} \leq 10$), even if the variability of M is relatively high. However, the SR relationship generally cannot be defined in species, such as teleosts, with high fecundities ($\mathcal{F}$ on the order of 10^6) if prerecruit M exhibits even moderate variability because small variations in M lead to large changes in survivorship. Thus, the concept of a simple deterministic SR relationship does not appear applicable to organisms with high fecundities. The use of fishery population models dependent upon such functions needs to be reexamined. Adaptive management strategies (i.e experimental manipulations of stock size) are likely to succeed in defining the form of the SR relationship only for stocks characterized by low recruitment variability.

La stabilité de modèles de populations de poisson dépend souvent de la forme de la relation stock–recrutement (SR) sous-jacente, malgré que cette relation ne peut généralement pas être définie à partir de données réelles sur les pêches. L'auteur a utilisé un simple modèle SR pour démontrer que la capacité de caractériser cette relation dépend du taux de mortalité naturelle (M) pendant la période de pré-recrutement, taux lié au taux de fécondité ($\mathcal{F}$) d'une espèce, et la variabilité de M. La relation SR peut être facilement établie chez les espèces à faible taux de fécondité (par ex., les vertébrés évolués dont $\mathcal{F} \leq 10$), même si la variabilité de M est relativement élevée. Toutefois, cette relation ne peut généralement pas être définie chez des espèces comme les téléostéens à taux élevé de fécondité ($\mathcal{F}$ de l'ordre de 10^6) si le M pendant la période de pré-recrutement varie même modérément étant donné que de légères variations de ce taux entraîne de grandes fluctuations du taux de survie. Par conséquent, la notion d'une simple relation SR déterminante ne semble pas applicable à des organismes montrant un taux élevé de fécondité. L'utilisation de modèles de populations exploitées qui dépendent de telles fonctions doit donc être réévaluée. Des stratégies de gestion flexibles (c.-à-d. des manipulations expérimentales de la biomasse du stock) n'auront du succès dans la définition de la forme de la relation SR que dans le cas de stocks caractérisés par une faible variabilité du recrutement.

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The stock–recruitment (SR) relationship is central to many models used to determine optimal fishing strategy, since this relationship defines the stock's ability to replenish itself as stock size is reduced by exploitation. Even when recruitment is assumed to vary substantially in relation to environmental variability (Getz et al. 1987; Koslow 1989), mean long-term sustainable yield appears to depend primarily upon the degree of compensation or density dependence in the SR relationship, i.e. the degree to which the stock may be reduced from its virgin state before recruitment is significantly impaired. Most successful commercial fisheries are characterized by mean recruitment being remarkably robust to variation over a wide range of stock sizes.

However, fishery SR data are typically so highly variable that even the shape of the relationship cannot be determined with confidence for particular stocks (i.e. whether it is weakly or strongly asymptotic; asymptotic or dome-shaped), despite the profound implications such distinctions may have in models of population dynamics. As a result, there is increasing reluctance among some fishery scientists to fit curves based on particular SR models (e.g. Beverton and Holt 1957; Ricker 1954; Shepherd 1982) to the apparently random scatter of SR data sets (Getz and Swartzman 1981; Evans and Rice 1988).

Why does the SR relationship seem so poorly defined in fisheries? Walters and Ludwig (1981) considered that it might be obscured by error in measurement of stock size. However, measurement error has not prevented fishery scientists from reporting a plethora of correlations between recruitment and environmental variables. Many workers assume that the SR relationship cannot be discerned due to the effects of environmental variability (Goodyear and Christensen 1984; Walters and Collie 1988), but statistical removal of the environmental influence on recruitment does not seem to lead to convincing SR relationships (Rounsefell 1958; Bailey 1981). Frustration with this state of affairs has led some modellers to call for "adaptive management" of fisheries (Smith and Walters 1981; Walters 1981; Walters and Collie 1988), i.e. experimental manipulation of selected stocks to examine recruitment at varying stock sizes and thereby to separate the confounding influences on recruitment of environment and stock size. However, experimental stock reduction entails significant risk, so such proposals highlight the basic question — is there in fact a definable SR relationship underlying the apparent scatter?

An alternate view arose out of a long-standing, more general controversy in ecology regarding the relative importance of density-dependent and density-independent mortality in population regulation (Andrewartha and Birch 1954; Hairston et al. 1960; Ehrlich and Birch 1967; Strong 1986). Many fishery scientists would concur with Strong (1986) that fish are typical of

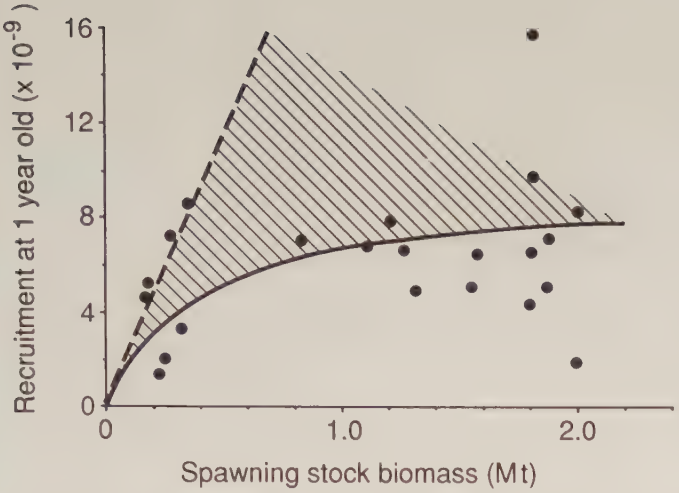

FIG. 1. Stock–recruitment (SR) data for North Sea herring and a proposed SR relationship based upon Shepherd (1982). The broken line indicates the apparent density-independent SR relationship, and the difference between this line and the SR relationship indicates losses due to density-dependent mortality.

populations that, except at extremes of population size, are regulated primarily by highly variable factors unrelated to stock size, i.e. predation and environmentally induced variability in advective losses and food availability. Based upon this argument, recruitment may be unrelated to stock size, or only weakly, linearly related to stock size, except when the spawning stock is exceptionally high or low. As Sissenwine and Shepherd (1987) proposed, the correct null hypothesis for this state of affairs may be that there is no compensation in the SR relationship, i.e. that R/S is constant over a wide range of stock sizes.

However, others have argued, based upon the same SR data sets, that there is strong evidence in fisheries of density dependence. As Gulland (1982) noted, the stability of recruitment to most fisheries is perhaps more remarkable than its variability, if we consider the exceptionally high fecundity of virtually all commercially exploited fish species (10^4–10^7 eggs per female) and the corresponding high mortality that reduces these numbers 3–6 orders of magnitude in the first year of life. The range of recruitment to commercial stocks is often within a factor of 10 and, with a few notable exceptions, generally within a factor of 100 (Hennemuth et al. 1980; Koslow 1984). Based upon this argument, most data in fishery SR data sets are on the relatively flat right-hand limb of the SR curve, where recruitment is stabilized by density-dependent processes. Density-independent recruitment is found only in the initial portion of the SR relationship, where S and R are approximately linearly related, but the data required to define this initial portion of the curve can only be found at very low stock sizes (Fig. 1). The biological basis of this SR relationship may be the large excess egg production that appears to be a general feature of fish reproduction, i.e. the difference between the "density-independent" or initial linear SR relationship and observed or mean recruitment levels (Fig. 1). Thus there may be evidence of density-dependent mortality over virtually the entire range of fishery stock sizes typically encountered. But then why is it generally not possible to define such a relationship with existing SR data?

I hypothesize that the lack of definable SR relationships in fish is a necessary consequence of their general life-history strategy, i.e. their high fecundity ($\mathcal{F}$) and correspondingly high (and necessarily varying) mortality rates (M) during the early life history. Given that mortality is an exponential process, even small deviations in M lead to such large changes in fish survivorship when M is large as to entirely obscure the SR relationship. On the other hand, survivorship of creatures with markedly lower fecundity and mortality during the prerecruit phase, such as most higher vertebrates, is not so affected by natural variability in M, so their SR relationship may be readily apparent. To examine this hypothesis, I will examine the sensitivity of a simple SR model (Beverton and Holt 1957) to values of $\mathcal{F}$ (and M) and to levels of random variability in M. The Beverton–Holt model was chosen because it explicitly considers the implications of density-dependent and density-independent mortality upon recruitment. This exercise should shed light on the nature of SR data and set theoretical limits on the uses to which these data may be put. Is it feasible to attempt to define fishery SR relationships using historical data, whether or not stock size is experimentally manipulated? In this paper, I examine whether it is possible to discern the SR relationship using simulation data generated from a simple SR algorithm in the presence of varying degrees of stochastic variability.

TABLE 1. Values of the parameters used in simulations of recruitment for populations with low and high fecundity, $\mathcal{F} = 10$ and 10^6, respectively, and with population regulation weakly or strongly density dependent (DI, density independent; DD, density dependent). See Methods for details of the model and explanation of the parameters.

	$\mathcal{F} = 10$		$\mathcal{F} = 10^6$	
	DI	DD	DI	DD
μ_1	1.8	0.7	12.9	0.2
μ_2	0.07	0.7	3.2×10^{-6}	4.6×10^{-5}
α	0.2	1.0	0.1	0.4
β	6.0	2.0	4.0×10^5	10^5

Methods

The Beverton–Holt SR model is based upon separating M during the prerecruit phase into density-independent (μ_1) and density-dependent (μ_2) components, such that $M = \mu_1 + \mu_2 N$, where N is the number of prerecruits. The change in numbers is then expressed as

$$(1) \quad dN/dt = -M \cdot N = -(\mu_1 + \mu_2 N)N.$$

The period from spawning to recruitment may be divided into several stages (e.g. egg, larval, and juvenile) for which mortality and survivorship are determined separately. However, for the sake of simplicity, I treat here the entire period until year-class strength is determined (t_r) as a single stage, for which the initial number (N_0) at time t_0 is the number of eggs (E) produced, as $E = N_0 = S \cdot \mathcal{F}$, where $\mathcal{F}$ is adult fecundity in numbers per unit stock biomass; and the number of recruits (R) or survivors at t_r is determined by integrating the previous expression:

$$(2) \quad R = N_{tr} = \frac{1}{\frac{\mu_2}{\mu_1}(e^{\mu_1 t_r} - 1) + \frac{e^{\mu_1 t_r}}{S \cdot \mathcal{F}}}.$$

In its usual formulation, Equation 2 is simplified by substituting $\alpha = \mu_2/\mu_1 (e^{\mu_1 t_r} - 1)$ and $\beta = e^{\mu_1 t_r}$, such that

$$(3) \quad R = \frac{1}{\alpha + \frac{\beta}{S \cdot \mathcal{F}}}.$$

TABLE 2. Total instantaneous mortality rates (M) per prerecruit stage, standard deviations (SD), and coefficients of variation (CV) derived from field surveys (data for northern anchovy and plaice) and from virtual population analyses and information on fecundity (cod and haddock). Comparable statistics (mean, SD, CV) are shown for $\log_{10}$-transformed cod and haddock recruitment data (R) (Mt), shown in parentheses. Stage duration (in days) is shown in parentheses. N = number of observations in the time series. Data sources are shown below.

Species	Stage (duration)	Total M (log R)	SD	CV	N
Northern anchovy	Egg (4.4)	1.58	0.54	0.34	13
(*Engraulis mordax*)[a]	Larval (14.6)	2.71	0.34	0.13	13
	Juvenile (346)	6.38	1.17	0.18	13
	Total (365)	10.67			
North Sea plaice	Egg/larval (35)	3.46	1.81	0.41	12
(*Pleuronectes platessa*)[b]	Larval (115)	4.54	2.12	0.52	5
	0-group (~90)	2.55	1.04	0.47	10
	Total	10.55			
Labrador cod	Prerecruit	13.44	0.75	0.06	19
(*Gadus morhua*)[c]	(4 yr)	(8.52)	(0.27)	(0.03)	
Georges Bank haddock	Prerecruit	14.71	1.76	0.12	29
(*Melanogrammus aeglefinus*)[d]	(1 yr)	(7.36)	(0.77)	(0.10)	

[a]Peterman et al. (1988).
[b]Egg and larval mortality: Harding et al. (1978): 0-group mortality: Lockwood (1980).
[c]Recruitment and stock size: Rice and Evans (1988); fecundity: May (1967).
[d]Recruitment and stock size: Clark et al. (1982); fecundity: Hodder (1963) and Hislop and Shanks (1981).

Parameter α contains μ_2 and is therefore treated as a coefficient of density dependence whereas β is determined only by μ_1 and is considered a coefficient of density-independent mortality. Values for the parameters μ_1, μ_2, α, and β (Table 1) were selected, first, to provide several surviving offspring per female at the end of the prerecruit period and, second, to define a SR curve with either weak or strong density dependence in the absence of variability in natural mortality. Weak density dependence is characterized by $\beta/\mathcal{F} >> \alpha$, since Equation 3 then approximates $R \simeq 1/(\beta/S\cdot\mathcal{F}) = S\cdot\mathcal{F}/\beta$, such that R varies quasi-linearly as a function of S. A strong density-dependent relationship is defined by $\alpha >> \beta/\mathcal{F}$, such that R in Equation 3 rapidly approaches an asymptote $1/\alpha$.

Density-independent mortality was simulated to vary randomly by multiplying μ_1 by r^* where r^* is a random normally distributed variable with mean = 1 and standard deviation (SD) and coefficient of variation (CV) = 0.05, 0.12, or 0.25. These levels of variability produced low, moderate, and high levels of variability in prerecruit mortality and recruitment that were comparable with those observed in marine fisheries (Table 2). It should be noted in Table 2 that the variability of mortality calculated from field surveys of successive life history stages seems greater than that obtained from virtual population analyses. This may be due to greater sampling error associated with field surveys, as well as to possible density dependence in mortality, such that exceptional mortality rates experienced during one stage of the early life history may be compensated during succeeding prerecruit stages. The mortality data listed in Table 2 are not exhaustive but represent time series from several groups of fishes and include species noted for relatively stable (e.g. Atlantic cod (*Gadus morhua*)) and highly variable (clupeoids and haddock (*Melanogrammus aeglefinus*)) recruitment histories.

Since r^* was incorporated into the exponential mortality term of Equation 2, the resulting recruitment varied log-normally, as often observed in fishery recruitment time series (Hennemuth et al. 1980; Koslow 1984). The random number series from which r^* was generated (Gottfried 1982) was identical in all simulations; only the level of variability (SD) varied. The coefficient of density-dependent mortality (μ_2) was assumed to remain constant. To examine recruitment over a range of stock sizes, 25 values for stock size (S) were selected at random from a uniform distribution of numbers between 0 and 2 (the scale is arbitrary). The same values for stock size were used in all simulations presented, along with the corresponding array of 25 values of r^*. The values of S cover a wide range of stock size, so the SR relationship is probably more easily defined here than from most historical SR data sets.

To calculate annual egg production, values of S were multiplied by $\mathcal{F}$ to obtain egg production. The model was examined for $\mathcal{F}$ ranging from 1 to 10^7, but results are presented here only for values of 10 and 10^6 eggs per female, i.e. fecundities on the order of those found in many higher vertebrates and in fish, respectively.

The ability to define the SR relationship in the simulated SR data was examined statistically. The relationship was linearized using an inverse transformation of Equation (3):

$$(4)\quad \frac{1}{R} = \alpha + \frac{\beta}{E}.$$

Ordinary least-squares regression of $1/R$ with $1/E$ was then used to determine the statistical significance and 95% confidence limits of the parameters α and β. Because the distributions of these variables were typically highly nonnormal, the significance of the relationship was also examined nonparametrically by testing the Spearman's rank-order correlation between $1/R$ with $1/E$.

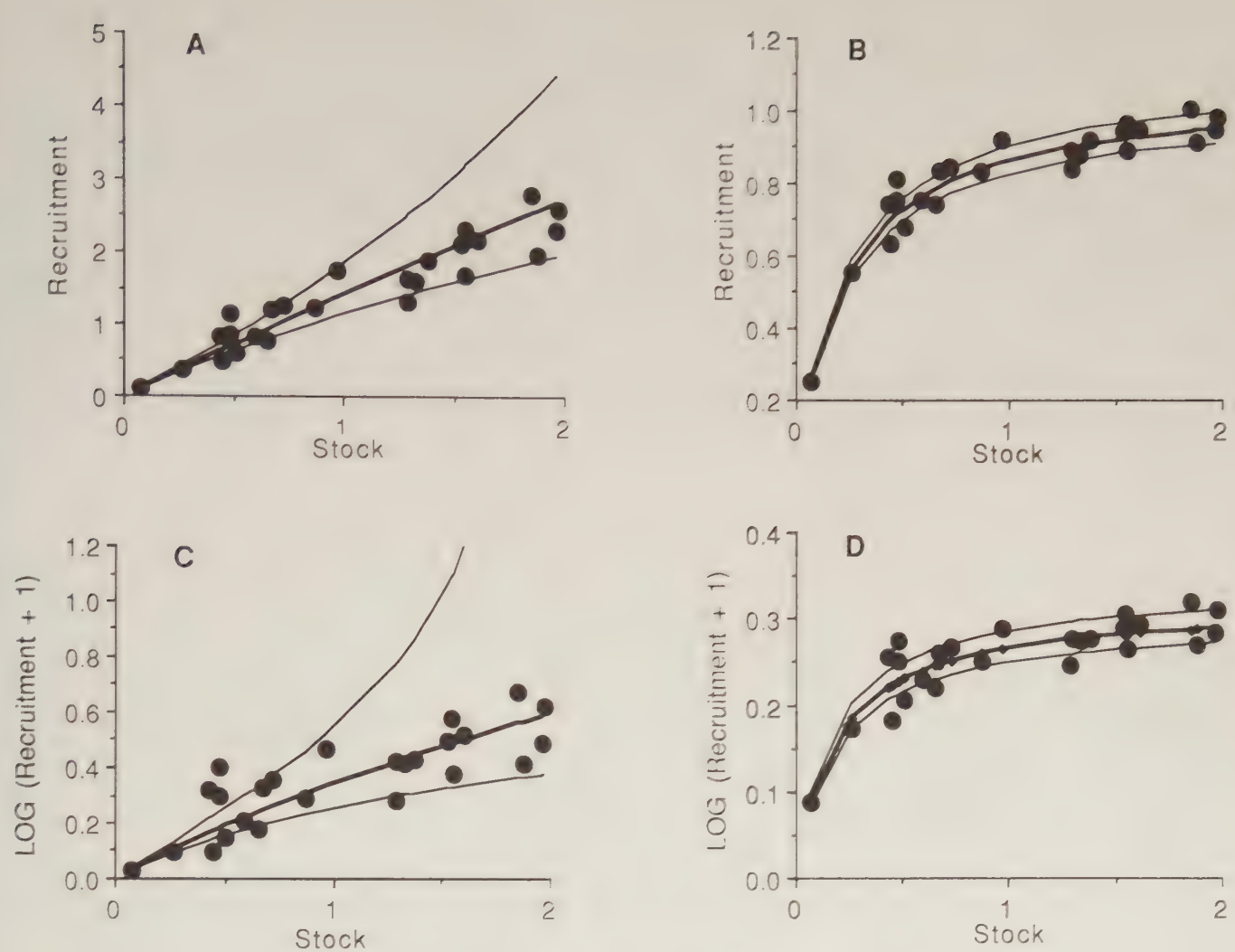

FIG. 2. Simulated stock (S) and recruitment (R) data for a population with fecundity $\mathcal{F} = 10$. Randomly generated values of S are the same for all simulations in Fig. 2 and 3. Recruitment is based upon the Beverton and Holt (1957) SR model (see Methods and Table 1 for details) with random variability added to the coefficient of density-independent mortality (μ_1) (Equation (2)). (A) Weakly and (B) strongly density-dependent SR relationship with SD (μ_1) = 0.12 and (C) weakly and (D) strongly density-dependent SR relationship with SD (μ_1) = 0.25. Note that the R data in Fig. 2C and 2D are log-transformed.

Results

The simulations indicate that the variability of the SR relationship for a population depends upon the species' life history strategy (i.e. the order of magnitude of its fecundity and corresponding mortality during the prerecruit phase), as well as the variability of prerecruit mortality (Fig. 2, 3). The simulated SR data were less variable and hence the SR relationship better defined for the population with fecundity typical of higher vertebrates ($\mathcal{F} = 10$) and high variability of natural mortality (CV (μ_1) = 0.25) (Equation (2)) (Fig. 2C, 2D) than for the model population with fecundity typical of teleost fish ($\mathcal{F} = 10^6$) with very low variability in mortality (CV (μ_1) = 0.05) (Fig. 3C, 3D). The parameter α, which defines the asymptotic level of recruitment and thereby the shape of the SR relationship (Equation (3)), was not determined significantly for any of the weakly density-dependent simulations with variable natural mortality during the prerecruit period. However, α could be defined for all simulations based upon a strong density-dependent SR relationship when fecundity was low ($\mathcal{F} = 10$), but only at the lowest level of variability in M (CV (μ_1) = 0.05) when fecundity was high (Table 3; Fig. 2, 3). No significant SR relationship could be defined for simulations of a high-fecundity population with highly variable natural mortality (CV (μ_1) = 0.25) (Table 3; Fig. 3G, 3H).

Discussion

The presence of a significant SR relationship may be detected in teleost fish populations when the variability in prerecruit mortality is moderate (CV (μ_1) < 0.12) (Equation (2)), i.e. when the range in recruitment is less than 2 orders of magnitude. However, the shape of the SR relationship is unlikely to be definable except under a restrictive set of conditions: when the relationship is strongly density-dependent and the variability of prerecruit mortality is very low (CV (μ_1) $\leq$ 0.05), i.e. when the range in year-class strength is less than an order of magnitude over a wide range of stock sizes. Even under these conditions, the confidence limits around the SR relationship are likely to be quite large (Fig. 3C, 3D).

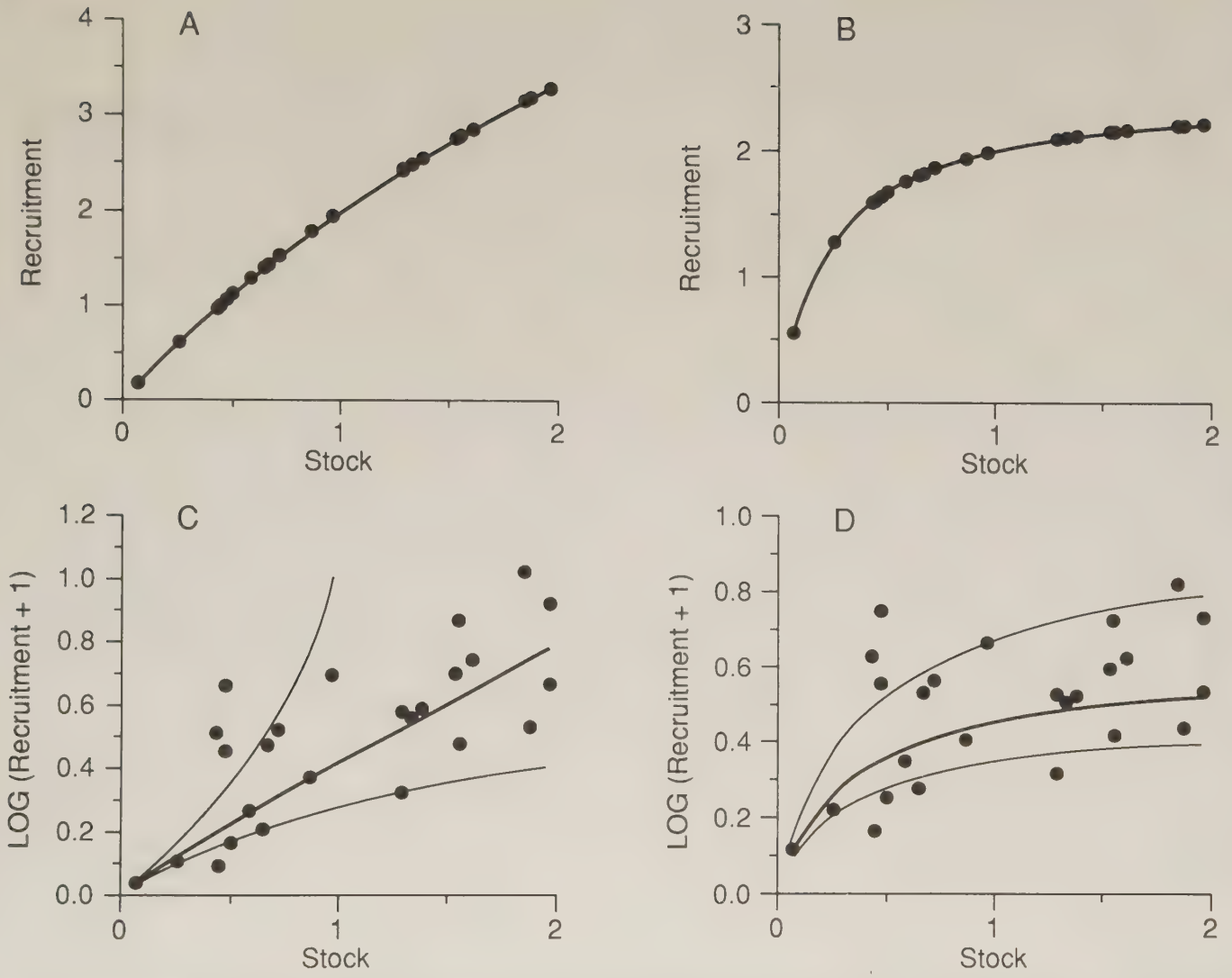

FIG. 3. Simulated stock (S) and recruitment (R) data for a population with fecundity $\bar{\phi} = 10^6$. Randomly generated values of S are the same for all simulations in Fig. 2 and 3. Recruitment is based upon the Beverton and Holt (1957) SR model (see Methods and Table 1 for details) with random variability added to the coefficient of density-independent mortality (μ_1) (Equation (2)). (A) Weakly and (B) strongly density-dependent SR relationship without added random variability, (C) weakly and (D) strongly density-dependent SR relationship with SD (μ_1) = 0.05, (E) weakly and (F) strongly density-dependent SR relationship with SD (μ_1) = 0.12, and (G) weakly and (H) strongly density-dependent SR relationship with SD (μ_1) = 0.25. Note that the R data in Fig. 3C–3H are log-transformed. (*Fig. 3 concluded next page*)

The basis of the strong influence of fecundity and its associated levels of natural mortality upon the SR relationship is that variability in natural mortality acts exponentially upon recruitment. Thus if the fecundity of commercial marine fish species generally lies between 10^4 to 10^7 eggs per female, on the order of 10–100 will survive to the end of the first year. This implies that an instantaneous mortality rate (M) (yr^{-1}) of 7–16 ± 25% variation in mortality leads to an approximate 30- to 3000-fold range in number of survivors per female. A similar calculation for higher vertebrates with 1–100 young per year indicates that at equilibrium, M = 0.7–4.6, and variability in survivorship per female (at ±25% variability) is only 1.4–10.

This exercise indicates that hypotheses concerning the SR relationship and population regulation by density-dependent versus density-independent processes are largely untestable for many, if not most, marine fish and invertebrate populations. Although recruitment in the model was determined by SR relationships exhibiting marked density dependence or independence, addition of variability in natural mortality obscured the form of the relationship, unless the variability of recruitment data was low due to strong density dependence and low variability in prerecruit mortality. The concept of a simple deterministic SR relationship, which may provide insight into the population dynamics of species with small fecundities, therefore appears to be of limited applicability when applied to organisms characterized by high fecundities.

The simulations indicate that adaptive management strategies (i.e. experimental manipulations of stock size) are likely to succeed in defining the form of the SR relationship only for stocks characterized by low recruitment variability. Of course, in dealing with actual fisheries, it may not be possible or desirable to obtain the wide variation in stock size that permits discrimination of the SR relationship even for stocks with relatively low levels of variability in prerecruit mortality. However, at moderate or higher levels of recruitment variability, a SR relationship is unlikely to be defined regardless of the extent to which stock size is manipulated.

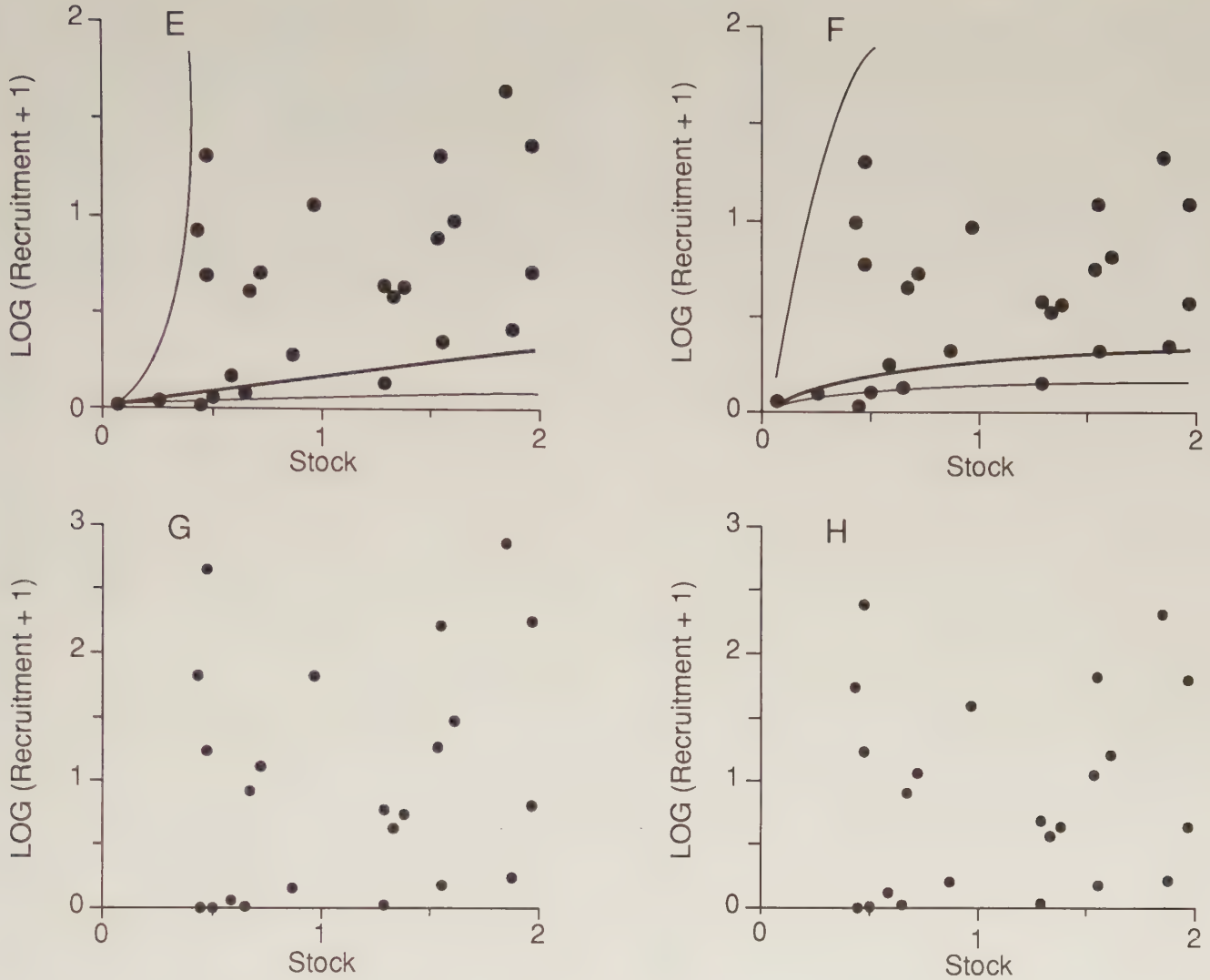

FIG. 3. (*Concluded*)

TABLE 3. Parameters (α and β) of the SR relationships shown in Fig. 2 and 3 and their significance based upon Spearman's rank correlation test and regression analysis carried out on the linearized, inverse-transformed data. $\mathscr{F}$; fecundity; DI, density-independent; DD, density-dependent; CV(M), coefficient of variation of mortality; ρ, Spearman's rho. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

Figure	$\mathscr{F}$	DI/DD	CV(M)	α ± SE	β ± SE	ρ
2A	10	DI	0.05	0.02 ± 0.06	0.69 ± 0.02***	0.94***
2B	10	DD	0.05	0.94 ± 0.02***	0.22 ± 0.005***	0.93***
2C	10	DI	0.25	−0.16 ± 0.16	1.00 ± 0.05***	0.84***
2D	10	DD	0.25	0.93 ± 0.04***	0.25 ± 0.01***	0.81***
3A	10^6	DI	0.00	0.10***	0.40***	1.00***
3B	10^6	DD	0.00	0.40***	0.10***	1.00***
3C	10^6	DI	0.05	−0.23 ± 0.19	0.84 ± 0.06***	0.76***
3D	10^6	DD	0.05	0.32 ± 0.10**	0.22 ± 0.03***	0.46*
3E	10^6	DI	0.12	−0.23 ± 1.57	2.31 ± 0.49***	0.30
3F	10^6	DD	0.12	0.57 ± 0.61	0.59 ± 0.19**	0.41*
3G	10^6	DI	0.25	43.06 ± 70.45	17.57 ± 22.25	0.44*
3H	10^6	DD	0.25	12.83 ± 17.60	3.80 ± 5.56	0.37*

Are SR models of use if the parameters cannot be defined? The parameters of existing SR models are not related to factors that actually regulate survivorship during the early life history, i.e. the spatial and temporal distribution of prey organisms, competitors, predators, and oceanographic features (Rothschild 1986; Beyer 1989). The processes regulating population size and its variability remain unknown for most fishes, and different processes may have quite different implications for popu-

lation stability and resilience to fishing and natural disturbance. It is unlikely, however, that further understanding will be gained through the continued use of inappropriate models.

In this regard, the processes that regulate recruitment to low-fecundity species may differ fundamentally from those regulating recruitment to marine fish and invertebrate populations characterized by very high fecundities. Higher vertebrates and other species characterized by low fecundity typically exhibit parental care and/or adult territoriality. The availability of reproductive territory provides a clear basis for a highly asymptotic relationship between recruitment and stock size. On the other hand, marine fish and invertebrate populations typically broadcast large numbers of very small reproductive products. From the vantage of the population, this results in substantial wastage of its reproductive output, i.e. the difference between the line projected from the origin of the SR relationship (the potential density-independent production) and the observed or mean annual recruitment (Fig. 1). This accounts for the resilience of fish populations to exploitation, i.e. the considerable degree to which the adult population may be reduced before reproductive potential is significantly diminished. This argument also indicates that density-dependent processes may operate across a wide range of stock sizes, and not only at high and low extremes of population size as indicated by Andrewartha and Birch (1954), Strong (1986), and others. However, although this argument seems to presuppose regulation of recruitment by density-dependent processes, other processes, such as prey-switching by predators as a function of prey density, may lead to similar recruitment patterns (Bailey and Houde 1989). Finally, it should be noted that this reproductive strategy provides resilience by spreading reproductive products over a spatially and temporally heterogeneous environment, thus providing opportunity for successful development wherever favorable habitats are found. The appearance of recruitment stability may thus arise from statistical averaging over this broad distribution of the variety of oceanographic processes (i.e. advection, the distribution of food, predators, and competitors) that regulate production and mortality in the sea.

Fresh approaches seem required in examining the sustainability of fisheries in relation to stock size. Existing models of fishery population dynamics based upon a SR function tend to show that the form of the relationship largely determines sustainable yield (Getz et al. 1987; Koslow 1989). However, fitting SR functions to available fishery data sets generally holds little promise of success; and similarly the likelihood is generally low that experimental reductions of stock size will further define the SR relationship and thereby compensate for the risk involved. The current practice of using long-term mean recruitment levels in management models ignores the variability and implicitly assumes a constant asymptotic recruitment relationship. Alternative approaches include recruitment forecasting based upon time-series and statistical or mechanistic modelling. Recruitment probabilities may also be estimated based upon the empirical probability distribution of recruitment at different stock levels (Getz and Swartzman 1981; Evans and Rice 1988). Alternatively, if the historical SR distribution is considered stationary, albeit indeterminate, it may be used to estimate a level of fishing at which the stock will replace itself (Sissenwine and Shepherd 1987).

Walters and Collie (1988) recently questioned whether research into the environmental influences on fishery recruitment was useful, since development of long-term management strategies depends rather upon defining the SR relationship. However, for many marine populations the SR relationship may not be definable or may also be defined with such broad limits as to be of little value as the basis for management. Research into recruitment processes may point the way toward more appropriate population models.

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Growth in Tropical Cephalopods: An Analysis Based on Statolith Microstructure

George D. Jackson and J. Howard Choat

Department of Marine Biology, James Cook University, Townsville, Queensland 4811, Australia

Jackson, G. D., and J. H. Choat. 1992. Growth in tropical cephalopods: an analysis based on statolith microstructure. Can. J. Fish. Aquat. Sci. 49: 218–228.

Validated size-at-age data are presented for two tropical squid species (*Loliolus noctiluca* and *Loligo chinensis*) and a sepioid (*Idiosepius pygmaeus*). Estimates of age were obtained from daily increments in the statolith. All species reached adult size in less than 200 d. For each species, growth in mantle length was linear over the sizes sampled. In *L. chinensis*, growth was fastest during December–January (summer), with males showing faster growth rates than females. For *I. pygmaeus*, females generally had a higher growth rate than males. The slowest growth rates for both sexes occurred in the August–September (winter) period. The size-at-age data indicated rapid linear or exponential growth and a short life span of less than 1 yr. In contrast, growth curves generated from analysis of length frequency data (ELEFAN software package) suggested an asymptotic growth curve and ages in excess of 3 yr, and such analyses therefore appear inappropriate. The results of this study and a review of the literature revealed that rapid growth and short life span is the norm for pelagic cephalopods, with tropical species growing considerably faster than their temperate counterparts.

On présente des données vérifiées sur la longueur selon l'âge de deux espèces tropicales de calmar (*Loliolus noctiluca* et *Loligo chinensis*) et d'une sépiole (*Idiosepius pygmaeus*). Les couches de croissance quotidienne identifiées sur les statolithes ont servi à l'estimation de l'âge. Les trois espèces atteignent la taille adulte en moins de 200 jours. L'augmentation de la longueur du manteau des trois espèces s'est révélée linéaire dans toutes les classes de longueur échantillonnée. Chez *L. chinensis*, la croissance était plus rapide en décembre et janvier (été): les mâles présentaient toutefois des taux de croissance plus rapide que les femelles, au contraire de chez *I. pygmaeus*. Chez cette dernière, les plus faibles taux de croissance des deux sexes ont été notés en août et septembre (hiver) Les données sur la longueur selon l'âge révèlent un taux rapide de croissance linéaire ou exponentielle et une durée de vie de moins de 1 an. Par contre, les courbes de croissance obtenues de l'analyse de données des fréquences de longueur (logiciel ELEFAN) indiquent une courbe de croissance asymptotique et des âges supérieurs à 3 ans. De telles analyses ne semblent donc pas appropriées. Les résultats de la présente étude et un examen d'ouvrages publiés ont révélé qu'une croissance rapide et qu'une courte durée de vie étaient la norme chez les céphalopodes pélagiques, les espèces tropicales présentant un taux de croissance plus rapide que leurs homologues des eaux tempérées.

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A topical subject of study is the comparison of demography and life histories of cephalopods and teleost fishes (Packard 1972; O'Dor and Webber 1986; Calow 1987). This requires properly validated estimates of age for pelagic cephalopods. Only with such data can we estimate age-specific schedules of growth, reproduction, metabolic activity, and life spans. This information is also of critical importance to the development of realistic models of growth and mortality for commercially fished cephalopods.

There is now an increasingly widespread use of statolith increments as a basis for estimating ages and growth rates of squids (Rodhouse and Hatfield 1990). Validated daily increments in statoliths provide a useful tool for determining growth rate and estimating its variance. A particular advantage of the cephalopod statolith is that daily increments may be reliably observed in the larger individuals, a strikingly different situation from that in teleosts (Hurley and Beck 1979; Kristensen 1980; Rosenberg et al. 1981; Hurley et al. 1985; Dawe et al. 1985; Natsukari et al. 1988; Jackson 1989, 1990a). However, opinions differ with respect to the adequacy of statolith-derived information as a basis for fisheries work. In a recent review, Boyle (1990) argued that there was insufficient information to allow for the development of growth models based on the analysis of statolith increments. Additional validation work and larger sample sizes are required.

Three main methods have been used to obtain estimates of squid growth rate and to infer longevities: analyses of length frequency data (e.g. Pauly 1985; Longhurst and Pauly 1987; Supongpan 1988), maintenance experiments in which growth is monitored in cultured individuals (Hixon 1980; Yang et al. 1983, 1986; Forsythe and Hanlon 1989; Hanlon et al. 1989), and size-at-age estimates derived from the analysis of statolith increments. There is an association between analytical method and the type of growth curve indicated. Length frequency analyses can produce asymptotic growth curves and relatively long life spans (see review in Mangold 1983; also Rao 1954; Nesis 1983; Worms 1983; Pauly 1985; Longhurst and Pauly 1987; Supongpan 1988). Size-at-age data and culturing experiments usually suggest rapid growth and short life span, with the form of the growth curve being linear or exponential (see review in Rodhouse and Hatfield 1990; Forsythe and Van Heukelem 1987).

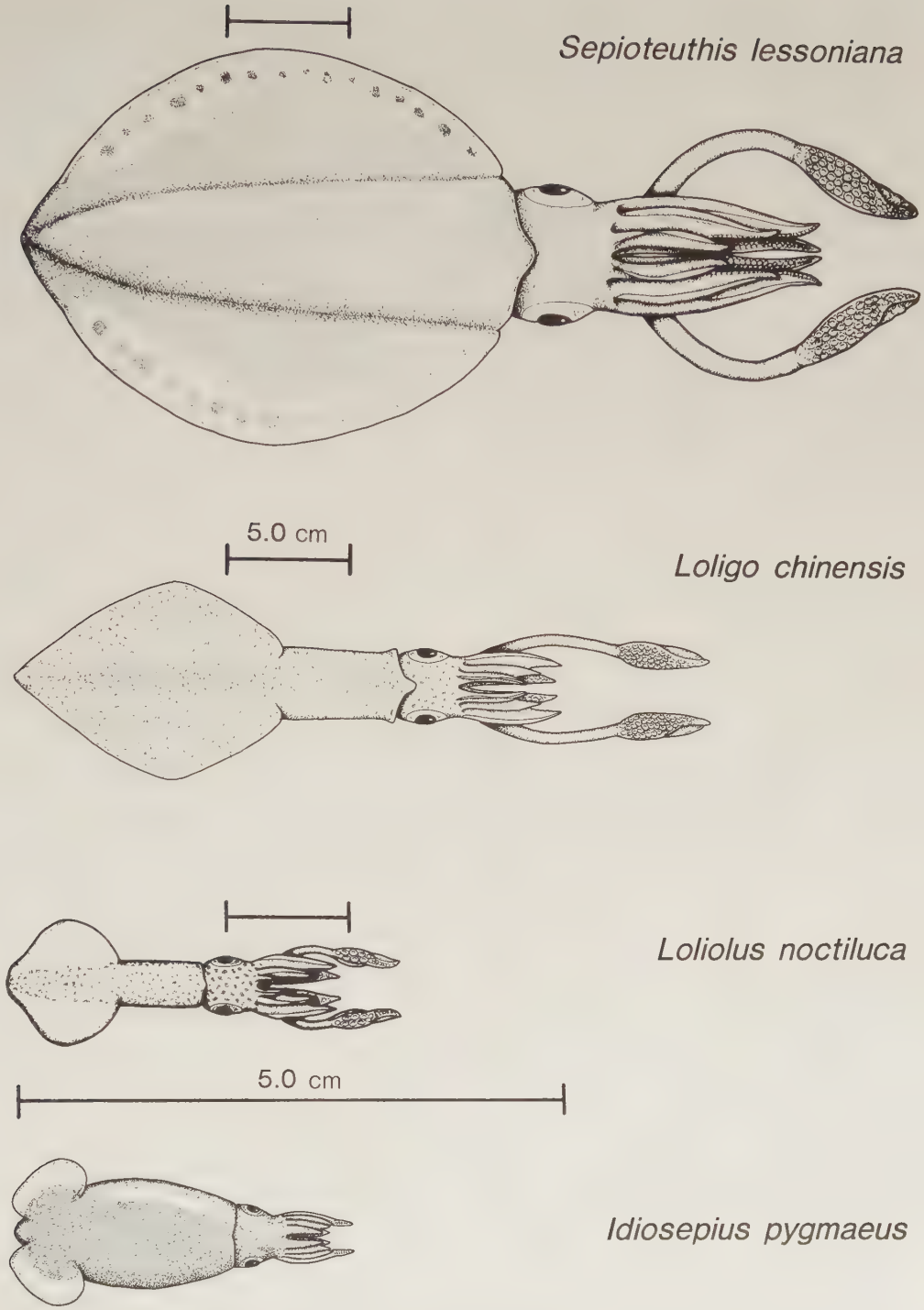

FIG. 1. The four species of cephalopods studied showing relative size differences. The three loliginid squids are mature males. Note the different scale for *I. pygmaeus*, which is a mature female.

Although some fisheries biologists have advocated that established growth models applicable to fin-fishes such as the von Bertalanffy function are the most appropriate way to describe cephalopod growth (e.g. Worms 1983; Pauly 1985; Longhurst and Pauly 1987; Supongpan 1988; Boyle 1990), other biologists have argued that growth functions commonly applied to fin-fishes are inappropriate for pelagic cephalopods (e.g. Forsythe and Hanlon 1989). If cephalopod demographic and life history patterns are considerably different to fish, they then require different approaches to the analysis of growth.

Cephalopod length frequency analysis may also be unsuitable for species which exhibit size- or age-dependent migrations out of the population (e.g. Dawe 1981; Dawe et al. 1981; Rowell et al. 1985; Patterson 1988) regardless of what model is applied to the data. Analysis of such data may incorrectly suggest that growth is asymptotic.

Results from the different methods of growth analysis tend to be confounded by the environment. As squid growth appears to be directly related to temperature (O'Dor and Wells 1987; Forsythe and Hanlon 1989), tropical species might be expected

TABLE 1. Comparison in *L. chinensis* of the number of increments extrapolated from submarginal areas of the statolith compared with the actual increments counted at the marginal area of equivalent width in clear statoliths. S, summer specimens; W, winter specimens.

Sex	Season	Mantle length (mm)	Age (d)	Extrapolated area of statolith radius (%)	Extrapolated increments from submarginal areas		Observed increment number in marginal areas	
					Nos.	$\bar{X}$	Nos.	$\bar{X}$
M	S	109	93	11.4	13, 14, 13	13	13, 14, 12	13
M	S	110	78	9.1	10, 10, 10	10	10, 11, 11	11
M	S	102	76	7.9	9, 10, 10	10	9, 9, 9	9
F	S	94	77	7.5	12, 11, 11	11	13, 13, 13	13
F	S	102	87	7.7	9, 9, 10	9	10, 10, 11	10
F	S	110	83	10.4	12, 11, 11	11	11, 12, 13	12
M	W	104[a]	119	7.0	11, 11, 11	11	12, 11, 13	12
M	W	109[a]	132	7.3	12, 11, 11	11	10, 10, 11	10
F	W	106[a]	119	6.1	11, 10, 10	10	10, 10, 10	10
F	W	108[a]	107	6.25	11, 11, 11	11	11, 11, 12	11

[a]Mantle length of fresh specimens.

TABLE 2. Maximum sizes attained for the study species and *S. lessoniana* based on our sampling program and as recorded from the literature.

Species	Maximum length (mm), this study		Maximum length (mm), recorded		Reference
Idiosepius pygmaeus	Male	10.3	Male	12.0	Voss 1963
	Female	17.6	Female	14.0	
Loliolus noctiluca	Male	58.0	Male	62.0	Lu et al. 1985
	Female	90.0	Female	83.0	
Loligo chinensis	Male	180.0		380[a]	Winstanley et al. 1983
	Female	120.0			
Sepioteuthis lessoniana	Male	213.0		360[a]	Roper et al. 1984
	Female	184.0			

[a]Sex not determined.

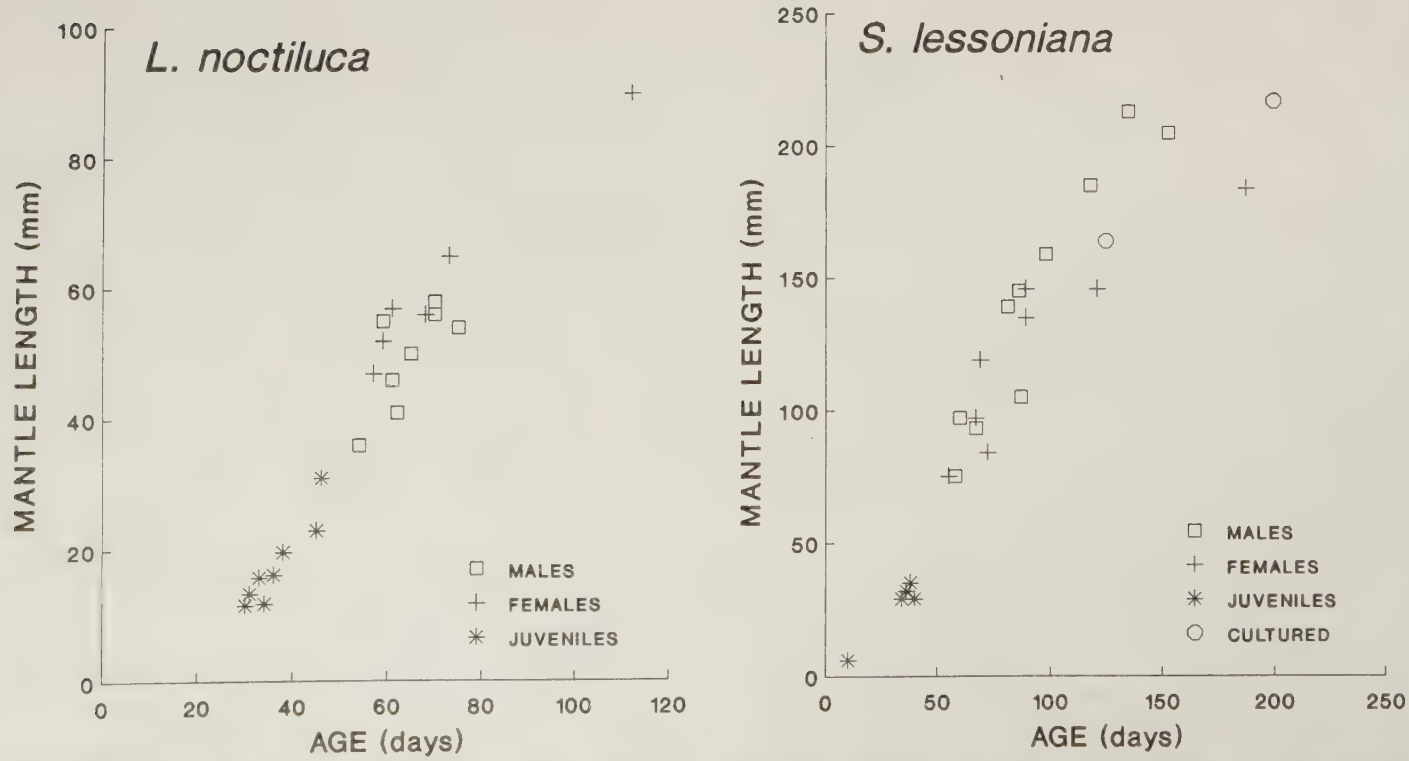

FIG. 2. Plots of length-at-age data for *L. noctiluca* and *S lessoniana*. *Sepioteuthis lessoniana* data from Jackson (1990a) with length-at-age determinations for two cultured individuals.

to grow more rapidly than those from temperate waters. However, as most estimates of growth in tropical squids are derived from length frequency analysis, while temperate and subtropical species have been studied using maintenance experiments, the question of the most appropriate method for studying cephalopod growth must be resolved.

In the present study, we provide descriptions of growth for four species of tropical pelagic cephalopod based on size-at-age data derived from statolith daily increments. For three of these species, we were able to compare these results with those obtained from length frequency analysis. This provided a basis for comparing growth in tropical and temperate squids.

Materials and Methods

Study Species

The three main cephalopod species used in this study represent two myopsid squids (*Loligo chinensis* and *Loliolus noctiluca*) from the family Loliginidae and one sepioid (*Idiosepius pygmaeus*) from the family Idiosepiidae, which includes some of the smallest of all the Cephalopoda (Fig. 1). Information on another species, e.g. *Sepioteuthis lessoniana* discussed by Jackson (1990a), is provided here for comparison with the other species and with material from cultured individuals. Our study species covered a wide length and weight range, with three orders of magnitude difference in weight between *I. pygmaeus* and *S. lessoniana*.

All four species of cephalopods studied were from tropical populations, although the distribution of the three myopsid squids extends into temperate waters. The distribution of *I. pygmaeus* includes northern Australia, Indonesia (Berry 1921), and the Philippines (Voss 1963). *Loliolus noctiluca* inhabits nearshore waters from New Guinea to Tasmania (Lu et al. 1985). *Loligo chinensis* is a wide-ranging species and is found in Australia from northern New South Wales and north to the South and East China seas and to southern Japan (Roper et al. 1984). The range of *S. lessoniana* extends across the top of Australia and down both the east and west coasts to subtropical waters. It also ranges from Hawaii to the Red Sea and north to Japan (Lu and Tait 1983).

Collection Methods and Sampling

The squids used in this study were all collected in waters off Townsville, North Queensland, with trawls, beach seines, and dip nets. The collection methods cover the full range of habitats of these squid species in the Townsville area. Individuals of *I. pygmaeus* which were aged and used for growth analysis were collected in March 1988 and 1989, May 1988, June 1988 and 1989, and August–September 1988. Individuals of *L. chinensis* which were aged and used in growth analysis were captured in summer (November–February) and winter (June–August), while individuals of *L. noctiluca* were captured between May and July 1989. Individuals of *L. chinensis* were initially preserved in 10% seawater–formalin buffered with borax and subsequently transferred to 70% ethanol within 2 d, while individuals of *I. pygmaeus* and *L. noctiluca* were preserved directly in 70% alcohol.

Surface water temperatures in the middle of Cleveland Bay have been shown to fluctuate seasonally between 19.3 and 30.9°C (Walker 1981) with summer maxima occurring in January and winter minima occurring in July. Temperature profiles across the continental shelf have shown little stratification (Milward and Hartwick 1986).

Length and Weight Measurements

Length measurements were taken as dorsal mantle length. Lengths of individuals of *I. pygmaeus* were measured with a stereo microscope eyepiece micrometer to the nearest 0.1 mm. Lengths of the larger squids were measured to the nearest millimetre. Wet weight was taken from preserved individuals which were blotted with paper towelling prior to weighing.

Statolith Analysis

Techniques employed for the validation, visualization, and counting of daily statolith growth increments have been described elsewhere for *I. pygmaeus* (Jackson 1989) and *S. lessoniana* (Jackson 1990a). The validation of daily growth increments in *L. chinensis* and *L. noctiluca* has also been described previously (Jackson 1990b). For three of the four species, daily periodicity in statolith growth increments has been demonstrated for adults. Only juveniles were available for the validation of *S. lessoniana*. However, the size range for the validated individuals of the other species were 7.6–14.4 mm for *I. pygmaeus*, 19.8–54 mm for *L. noctiluca*, and 81 and 111 mm for the two male *L. chinensis* collected, which were both mature.

In this study, specimens of *L. chinensis* preserved for longer than 2 mo tended to have statoliths with opaque margins, although 61% of the statoliths could be read in their entirety. For the remainder, growth increments in marginal areas had to be extrapolated. To validate this procedure, we counted increments in the marginal areas of clear statoliths and compared these with the increments in equivalent widths adjacent to marginal areas. The number of rings was similar in each of the areas counted. There was no evidence that the pattern of spacing in marginal areas differed from that in submarginal areas (Table 1). This was done for both summer and winter samples. For the 39% of statoliths with opaque margins, increment number was extrapolated from equivalent widths of submarginal areas. The actual area of marginal extrapolation was small, consisting of approximately 6–16% of the total statolith radius from the nuclear area to the edge in all statoliths treated this way.

Length Frequency Analysis

Length frequencies from *I. pygmaeus* and *L. chinensis* were analysed to derive growth curves using ELEFAN software (version 1.02) (see Pauly and David 1981). For *L. chinensis*, both sexes were pooled for the analysis and size class interval set at 10 mm. Sexually dimorphic male and female *I. pygmaeus* were analysed separately with the size class interval set at 1 mm. Length frequency data for *S. lessoniana* from the Indian beach seine fishery (Rao 1954, p. 47) were also analysed with ELEFAN. The growth curve with the best fit (i.e. the curve with the highest R_n value) was obtained by manipulating parameters such as L_{max} and K. The collection of *L. noctiluca* were not sufficient to carry out length frequency analysis.

Results

Maximum Size

Adult sizes of the four species spanned an order of magnitude (Table 2). *Idiosepius pygmaeus* was much smaller than the

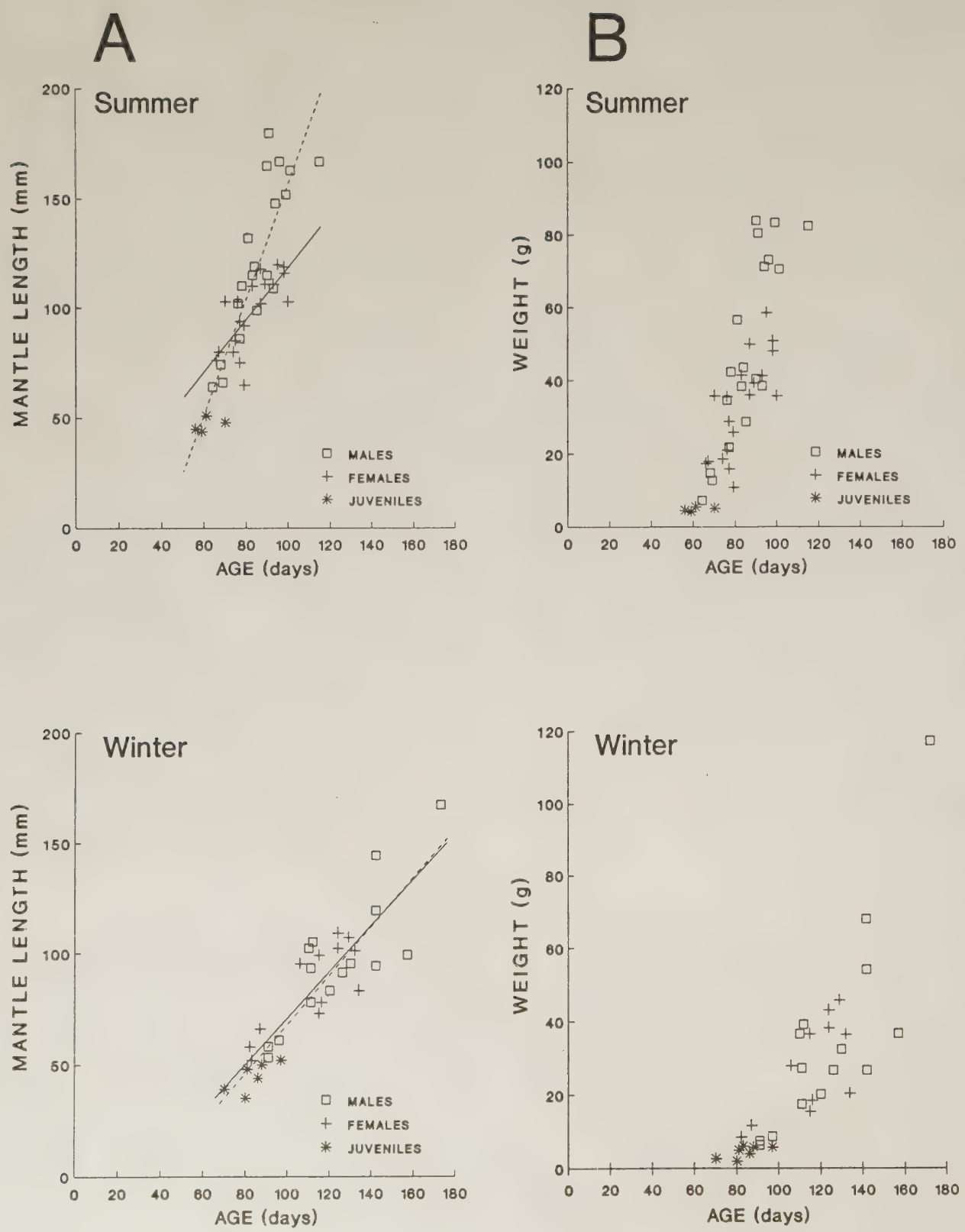

FIG. 3. Size-at-age data of *L. chinensis* collected in austral summer (January) and winter (June). Lines are least squares regressions calculated for each sex (broken lines = males, solid lines = females). (A) Length-at-age; (B) weight-at-age.

TABLE 3. Linear regression equations for the length-at-age data of male and female *L. chinensis* sampled during January (summer) and June (winter).

Season	Sex	n	Equation	r^2	p
Summer	Male	25	$y = -107.32 + 2.65x$	0.81	≤ 0.001
	Female	19	$y = -0.22 + 1.19x$	0.52	<0.001
Winter	Male	18	$y = -40.97 + 1.10x$	0.80	≤ 0.001
	Female	16	$y = -32.29 + 1.04x$	0.67	≤ 0.001

TABLE 4. Mean growth rates for summer and winter samples of male and female *L. chinensis*.

Season	Sex	Mean growth (mm/d)	±95% CI	No. of days	Size range (mm)
Summer	Male	2.65	0.54	59	74–180
	Female	1.19	0.59	35	69–118
Winter	Male	1.10	0.30	103	51–167
	Female	1.04	0.43	57	52–109

other species, with females attaining mantle lengths of up to 17.6 mm. The largest individual of *L. noctiluca* was a 90-mm female. *Sepioteuthis lessoniana* and *L. chinensis* both had mantle lengths greater than 100 mm. The largest sizes recorded in this and previous studies were 213 (Jackson 1990a) and 180 mm, respectively. Both these large individuals were males.

Size-at-Age Relationships

The size-at-age data used in this study covered the full size range that our sampling could detect. While it is possible that larger individuals of *L. chinensis* and *S. lessoniana* were present in the study area, it is unlikely in the case of *I. pygmaeus* and *L. noctiluca*. In these small nearshore cephalopods, we were able to sample the habitat comprehensively, and in the case of *L. noctiluca* the 90-mm specimen was the largest recorded for this species. The maximum recorded sizes of *S. lessoniana* and *L. chinensis* were considerably larger than those recorded from this study and from Jackson (1990a) (Table 2). However, the maximum lengths for these two species were similar to those obtained from other studies (Chan and Karim 1986; Rao 1954) in which very large individuals were rare.

Over the lengths sampled, the relationship between age in days and mantle length was linear for all species (Fig. 2–4). For *L. noctiluca* and *S. lessoniana* (Fig. 2), a possible curvilinear relationship was due to a single large individual in each case. If large individuals of *L. chinensis* and *S. lessoniana* were included in our samples an asymptotic plot might have resulted, but none was captured. Length-at-age data for two cultured individuals of *S. lessoniana* supplied by Dr. R. Hanlon (pers. comm.) (Fig. 2) conform to the growth curve derived from our sampled populations.

Sexual and Seasonal Growth Patterns of *L. chinensis*

There is considerable within-species variation in the recorded size-at-age relationships of cephalopods (Yang et al. 1986; Natsukari et al. 1988; Hanlon et al. 1989; Kinoshita 1989; this study). Two potential sources of within-species variation are sex-specific growth patterns and seasonal changes in growth rate. Male and female *L. chinensis* length-at-age linear regression for summer and winter samples (Fig. 3A) were highly significant (Table 3). Exponential or logarithmic regressions did not provide a better fit. Our estimate of growth (millimetres per day) over the length ranges sampled is the coefficient (slope) from the equation for linear regression of mantle length (millimetres) on age (days) (Table 4). There was evidence of both season- and sex-specific growth patterns. the most rapid growth occurred in the summer sample of males, which was considerably greater than that of the females from the same season. Winter samples indicated similar growth rates for males and females which were lower than growth rates for either sex in summer.

Covariance analysis of the regression data (Fig. 3; Table 5) was used to examine the effects of sex and season on growth. This revealed a significant difference in the data matrix for sex by season. The slope of the regression line for summer samples of males was significantly greater than those from the other combinations of sex and season. Comparisons of regression slopes among (1) male and female winter samples and (2) winter and summer female samples revealed no significant differences. Testing of elevations for the same combinations identified a significant difference between winter and summer females, with the latter displaying a substantially greater adjusted mean size (summer, 110.7 ± 3.4 mm S_x; winter, 64.4 ± 3.8 mm S_x). For winter samples, no significant differences could be detected in the rate or pattern of growth between males and females (Table 5). Males from the winter samples attained a greater age, but this may be a sampling artefact.

Data for the regression analysis were obtained from single periods during the summer and winter. To examine the generality of these results, we analysed two samples taken from within each seasonal period (summer and winter). This provided an opportunity to compare the magnitude of within- and among-season variation in length-at-age. As the numbers and size range of *L. chinensis* from each sampling period were not sufficient to allow for a balanced analysis of covariance, we used analysis of variance on the age estimates of squids from a predetermined size range (Table 6).

The data were analysed by a two-factor analysis of variance with sampling times nested within seasons. The only significant

TABLE 5. Summary of comparisons of regression equations based on analysis of covariance. **$0.01 \geq p > 0.001$, ***$0.001 \geq p$, ns = not significant.

Comparisons	Slope (F)	Elevation (F)	p	Significance
Summer, male vs. female	10.3		0.0027	**
Winter, male vs. female	0.07		0.795	ns
		0.08	0.777	ns
Female, winter vs. summer	0.209		0.650	ns
		62.72	0.0001	***
Male, winter vs. summer	26.43		0.0001	***

TABLE 6. Mean length and age ± SE of *L. chinensis* between 90- and 110-mm mantle length for males and females (n = 44) collected at two times within each season. The age data were subjected to analysis of variance. Sex and season are fixed factors and times within season a random factor. Variable is age within the specified size class.

	Summer		Winter	
	T_1	T_2	T_1	T_2
Females				
$\bar{X}$ length	102.2 ± 3.3	95.2 ± 1.5	100.5 ± 2.5	100.0 ± 2.1
$\bar{X}$ age	80.7 ± 2.6	98.7 ± 3.5	120.5 ± 6.1	123.2 ± 2.6
Males				
$\bar{X}$ length	105.0 ± 2.7	97.8 ± 1.6	98.2 ± 2.5	100.2 ± 1.8
$\bar{X}$ age	83.0 ± 3.8	90.0 ± 7.3	132.2 ± 9.5	126.0 ± 2.3

Analysis of variance table for age data (*0.05 ≥ p > 0.01; ns = not significant)

Source	df	ms	F	p	Significance
Sex	1	1.12	0.02	0.913	ns
Season	1	13592.49	39.66	0.024	*
Times (Season)	2	342.72	4.70	0.176	ns
Sex × Season	1	122.14	1.67	0.325	ns
Sex × Times (Season)	2	72.94	0.58	0.563	ns
Residual	36	124.78			

term in the model was "season" which reflected the higher growth rates of squids collected during the summer period. The term "times" was nonsignificant, although the power of this test was low (Table 6). Further subsampling within each season would undoubtedly reveal greater within-season variability in length-at-age. Despite this within-season variation in length-at-age, there was a clear seasonal trend.

Weight-at-age data were plotted for *L. chinensis* (Fig. 3B). Extrapolation of the weight-age relationship to lower size ranges suggests an extended period with little change in weight. Thus, growth was slow in the juvenile period with an abrupt increase in weight in the adults. This abrupt increase in weight is coincident with reproductive growth (G. D. Jackson, pers. obs.). If the curves were extrapolated to the approximate weight at hatching (approximately 0.78 mg, G. D. Jackson, unpubl. data), change in weight per unit time would be adequately described by an exponential relationship between weight and age. This relationship was similar to the age–weight relationship in *I. pygmaeus* (Jackson 1989).

Sexual and Seasonal Growth Patterns of *I. pygmaeus*

In the case of *I. pygmaeus*, samples were insufficient for comparisons between sexes and sampling times. The relationship between length and age was linear over the length ranges examined (Fig. 4). Fitting exponential equations to the female length-at-age data did not improve the fit. Data were available for four time periods: March, May, June, and August–September (Materials and Methods). The plots of the length–age relationship suggested (1) that for older sepioids, females at a given age were larger than males and attained larger sizes, and (2) time-dependent variation in growth with the slowest growth rates occurring in the August–September period. Regression analysis also identified considerable variation in the length-at-age relationship for both sexes during all seasonal periods. All regressions were significant with the exception of male *I. pygmaeus* in May (Table 7).

Covariance analysis was not attempted on this data set because of the substantial variation in several of the regression relationships. However, the mean growth rate data indicated that in all time periods except March, females grew faster than males (Table 7). For females the greatest growth was recorded during the periods of May and June. For males the rate of growth was greatest in March and lowest in August–September. A more robust analysis with a larger sample size would be needed to confirm these trends.

Length Frequency Analysis

Growth curves generated by length frequency (ELEFAN) analysis of samples of *I. pygmaeus* and *L. chinensis* (Fig. 5) were different from those obtained by statolith growth increment analysis. These data were based on length measurements of 847 *I. pygmaeus* captured over a 6-mo period and 2090 *L. chinensis* collected over a 12-mo period. The goodness of fit (R_n values) for the preliminary ELEFAN analysis ranged from relatively low values of 0.227 for *I. pygmaeus* females (age estimate of greater than 10 yr) to the highly significant value of 0.605 for *I. pygmaeus* males. Formal analysis was carried out on male *I. pygmaeus* and male and female *L. chinensis*.

The growth curve for *I. pygmaeus* males suggested a life span of 2.5–3 yr. For *L. chinensis*, a similar growth curve and a life span of 4–5 yr for the largest individuals in our study were suggested. For comparative purposes, we included an analysis of *S. lessoniana* length frequency data from Rao (1954). This provided a similar asymptotic curve with an estimate of time taken to reach 200-mm mantle length of 6–7 yr.

Discussion

Size-at-age data collected for these four tropical cephalopod species suggests (1) a life history pattern characterized by rapid growth, (2) no evidence of an asymptote in the growth curve, and (3) a comparatively brief life span. These characteristics were observed in four species which covered a wide size range. Our findings support the arguments of Forsythe and Van Heukelem (1987), Saville (1987), and Forsythe and Hanlon (1989) that asymptotic growth functions are inappropriate for describing cephalopod growth. The comparisons between the length-at-age plots and the length frequency analyses in this study confirm these views and suggest that length frequency analysis is unreliable for estimating growth and longevity in cephalopods.

The development of large-scale culturing programs for cephalopods has provided a means for corroborating estimates of demographic processes obtained from statolith analysis. However, the high variability of squid growth rates and their apparent sensitivity to environmental influences highlight some problems in the comparison of wild and cultured populations (Mangold 1983). Growth rates from the field may be less than those obtained from cultured poulations (Yang et al. 1983, 1986). Culture environments may also extend the life of cephalopods (Hanlon et al. 1989).

Length- and weight-at-age data from cultured cephalopods have provided growth curves which have been partitioned into exponential (juvenile) and logarithmic (adult) phases (Yang et al. 1986; Forsythe and Van Heukelem 1987). Our data for *L. chinensis* during summer suggest exponential growth rates over the full size range. The slowing of adult growth resulting in the "logarithmic growth phase" of Yang et al. (1986) and

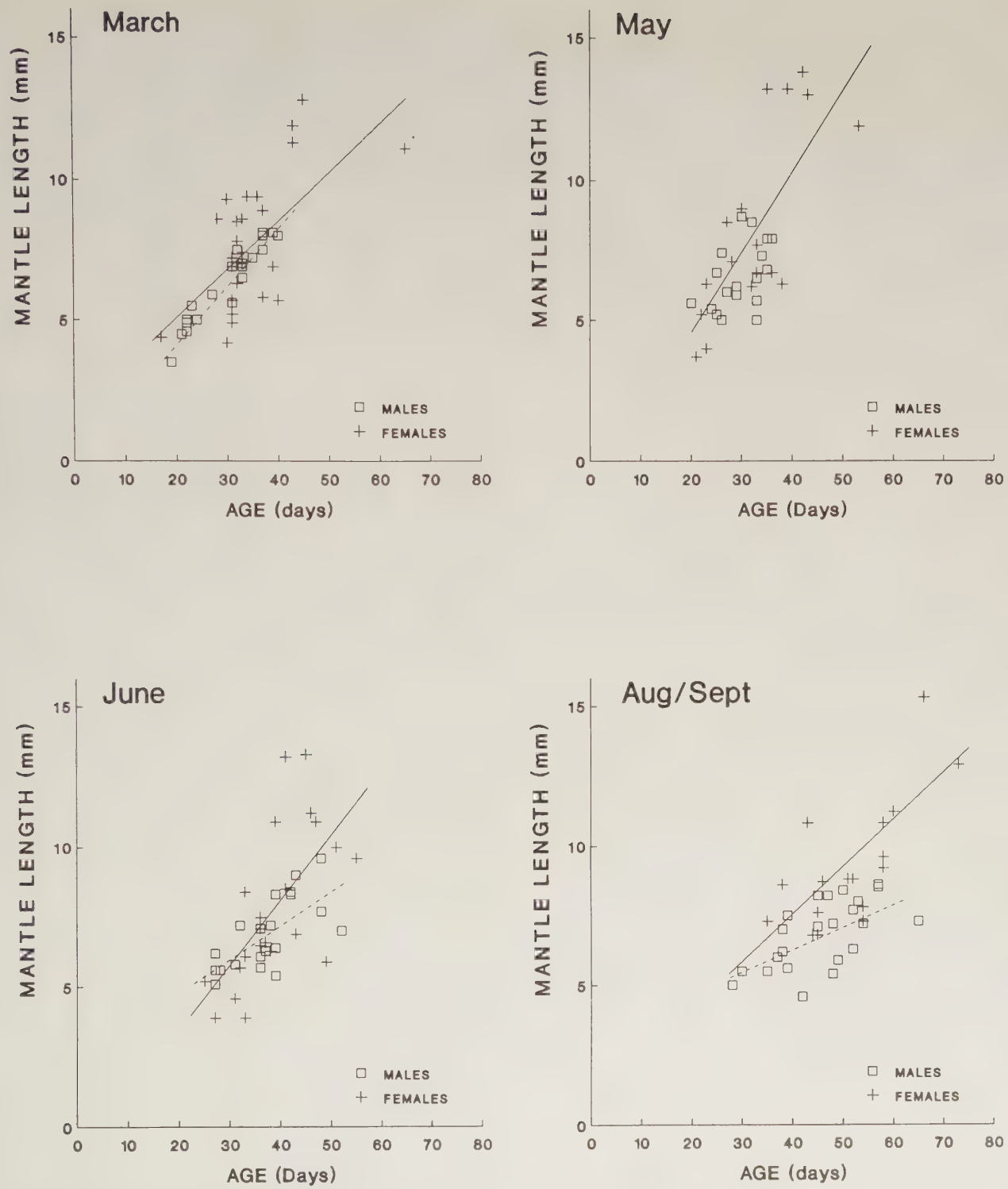

FIG. 4. Length-at-age data of *I. pygmaeus* from four time periods. Lines are least squares regressions for each sex (broken lines = males, solid lines = females).

Forsythe and Van Heukelem (1987) was not detected in species such as *L. chinensis* for which we had an extensive data set. This growth phase may be characteristic of culture conditions, or may not be observed in the wild due to migration of larger individuals.

Validation of daily increments in the present study species has been previously carried out on relatively large individuals (Jackson 1989, 1990a, 1990b). In this context the daily rings of squids are a more reliable ageing tool than in longer lived teleost fishes where explicit demonstrations of true daily increment formation in large individuals are problematic (Beamish and McFarlane 1987). There is still a requirement to continue validation work on statolith increment formation across as great a range of squid sizes and habitat conditions as possible. It is especially important to determine the pattern of statolith ring formation in cultured versus natural populations of squids where

TABLE 7. Regression equations and mean growth rates from the length-at-age data of male and female *I. pygmaeus* sampled from different seasonal periods.

Period	Sex	n	Equation	r^2	p	Mean growth (mm/d)	±95% CI
March	Male	22	$y = 0.53 + 0.19x$	0.86	<0.001	0.195	0.037
	Female	25	$y = 1.72 + 0.17x$	0.39	<0.001	0.174	0.093
May	Male	18	$y = 2.96 + 0.12x$	0.22	ns	0.121	0.119
	Female	17	$y = -1.27 + 0.29x$	0.56	<0.001	0.294	0.141
June	Male	20	$y = 2.35 + 0.12x$	0.47	<0.01	0.122	0.064
	Female	22	$y = -1.09 + 0.23x$	0.41	<0.01	0.230	0.128
Aug.–Sept.	Male	23	$y = 3.02 + 0.08x$	0.39	<0.01	0.083	0.047
	Female	17	$y = 0.74 + 0.17x$	0.51	<0.001	0.167	0.086

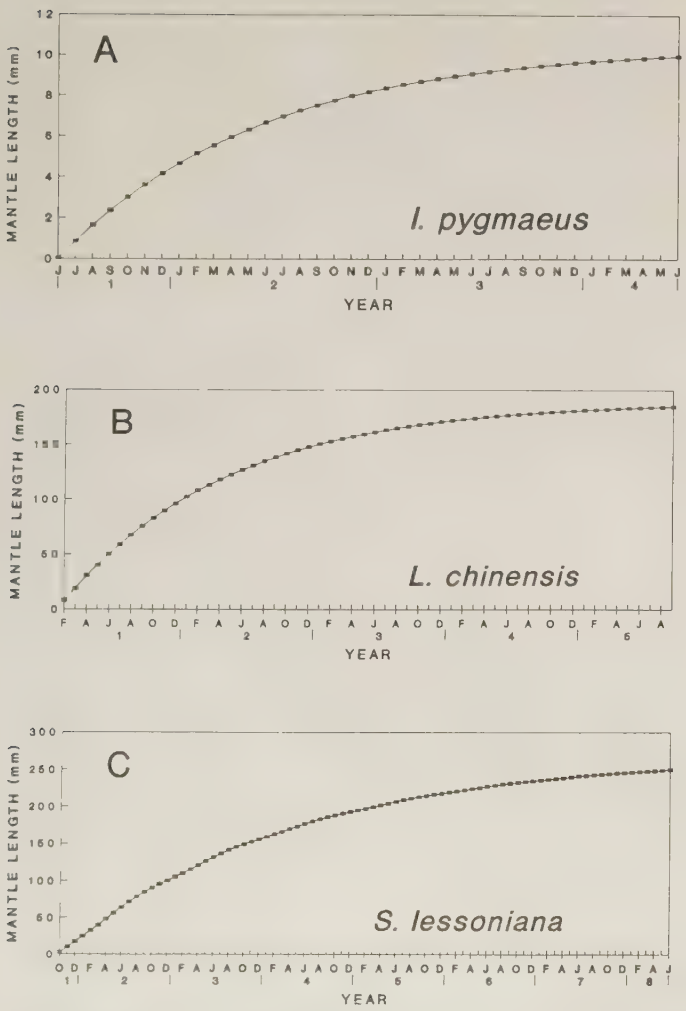

FIG. 5. Growth curves constructed from ELEFAN analysis of squid length frequency data. (A) Male *I. pygmaeus*: $K = 1.0$, $L_{max} = 10.5$ mm, $R_n = 0.605$. (B) *Loligo chinensis*: $K = 0.8$, $L_{max} = 186$ mm, $R_n = 0.308$. (C) *Sepioteuthis lessoniana* (data from Rao 1954): $K = 0.4$, $L_{max} = 268$ mm, $R_n = 0.322$. Note that the curve has a slight seasonal oscillation with a winter point of 0.9 and an amplitude set at 0.15.

systematic differences in feeding and environmental regimes must occur. This is also the need to establish at what stage in the life cycle increment formation begins (i.e. whether increments are present at hatching) to avoid overestimating age.

The problem of establishing estimates of mean life span in squid populations under natural conditions is a troubling one. Our data for the larger species suggest very short life spans (<200 d) and abrupt episodes of mortality for large individuals. We are aware that for *L. chinensis* and *S. lessoniana*, much larger specimens have been recorded than we obtained. Would a more comprehensive sampling program reveal substantial populations of large individuals and thus modify our estimates of growth and longevity? More extensive sampling of these species in other localities has demonstrated that the modal and upper sizes are similar to those obtained in this study (Rao 1954; Chan and Karim 1986) and that large individuals are rare.

Squid appear to have an extended juvenile period of growth which is especially evident in the weight-at-age plots for *L. chinensis*. Despite a rapid increase in size during the reproductive phase, squids appear to spend a large proportion of their lives as small individuals. However, care should be taken to ensure that subdaily increments or increments already present at hatching are not inflating the age estimates for small individuals. The suggestion of an extended juvenile growth phase contrasts with current life history theory which suggests that young cephalopods are thought to grow rapidly through a vulnerable juvenile period (Calow 1987).

Our data permit some comparisons between tropical and higher latitude squid populations. For both cultured (Forsythe and Van Heukelem 1987; Yang et al. 1986) and wild populations analysed for statolith increments (Kristensen 1980; Rosenberg et al. 1981; Natsukari et al. 1988), temperate-water squids appear to have slower growth rates and longer lives than tropical species.

The best growth estimates from the length frequency analyses indicated slow growth and a perennial life history. This is in contrast with the size-at-age data which indicate rapid growth and achievement of full size in months rather than years. We reviewed the literature to obtain information on growth estimates of both tropical and temperate squids. Although a number of growth estimates were available, comparisons were difficult due to the different methods used (e.g. growth estimates are sometimes averaged over an entire life span, while in other analyses, growth rates refer to growth only over a specified size range). We examined estimates obtained by length frequency analysis, maintenance experiments, and counts of statolith growth rings. There was also a single estimate based on mark–recapture (Araya 1983).

The greatest growth rates (Table 8) recorded were from statolith ageing of *Todarodes sagittatus* (2.5 mm/d, Rosenberg et al. 1981), *Photololigo edulis* (5.0 mm/d, Natsukari et al. 1988)

TABLE 8. Recent published information on squid life spans, along with information on growth rates[a] for some species. All species are from temperate populations except for *Stenoteuthis oualaniensis* (tropical) and *Loligo pealei*, *L. plei*, and *Lolliguncula brevis* (from subtropical populations).

Species	Life span (yr)	Method	Growth rate (mm/d)	Reference
Alloteuthis subulata	~1	Length frequency		Rodhouse et al. 1988
Loligo gahi	1	Length frequency		Patterson 1988
Photololigo edulis	1	Statoliths	5.0	Natsukari et al. 1988
Loligo pealei	1–1.5	Length frequency	0.82	Hixon 1980
		+ culture	0.87	
Loligo plei	1–1.5	Length frequency	0.67	Hixon 1980
		+ culture	1.97	
Lolliguncula brevis	1–1.5	Length frequency	0.71	Hixon 1980
		+ culture	0.42	
Loligo forbesi	1–2	Culture		Hanlon et al. 1989
		Length frequency	0.83	Holme 1974
Loligo opalescens	<1	Culture		Yang et al. 1986
Sepioteuthis lessoniana	~1	Culture	1.8	Segawa 1987
Illex illecebrosus	1	Statoliths		Radtke 1983
Gonatus fabricii	1.5–2	Statoliths		Kristensen 1980
Todarodes sagittatus	1.5	Statoliths	2.5	Rosenberg et al. 1981
Todarodes pacificus	~1	Mark–recapture + fishery statistics		Okutani 1983
Todarodes eblanae		Length frequency	1.47	Dunning and Brandt 1985
Stenoteuthis oualaniensis		Length frequency	0.8	Suzuki et al. 1986
Ommastrephes bartrami	~1	Mark–recapture	2.7	Araya 1983
Nototodarus sloani		Length frequency	1.33	Mattlin et al. 1985

[a]Growth rates were either given in the paper or calculated from information given in the paper. Growth rates from ageing and maintenance were calculated as average daily growth rate, based on known length-at-age. Growth rates from length frequency data were calculated from modal progression over time.

Loligo plei culture (1.97 mm/d, Hixon 1980), and mark–recapture experiments with *Ommastrephes bartrami* (2.7 mm/d, Araya 1983). Most of the recorded growth estimates were obtained from length frequency analysis. While these included a number of growth rates above 1.0 mm/d, ranging from 1.33 mm/d (*Ommastrephes bartrami*, Araya 1983; *Nototodarus sloani*, Hatanaka et al. 1985) to 1.50 mm/d (*Nototodarus* sp., Mattlin et al. 1985) to 1.67 mm/d (*Todarodes eblanae*, Dunning and Brandt 1985), the majority of estimates were below 1.0 mm/d. These included growth rates for *Loligo pealei* (Hixon 1980), *Loligo forbesi* (Holme 1974), and *Stenoteuthis oualaniensis* (Suzuki et al. 1986).

Our estimates of growth rate and size structure of tropical cephalopod populations suggest short life spans, of considerably less than 1 yr. This may reflect a sampling bias in that larger and older members were not collected by our methods. This is especially true for *L. chinensis* and *S. lessoniana*. However, an examination of the recent literature (Table 8) suggests that life spans of between 12 and 24 mo are the norm for pelagic cephalopods. Our results are consistent with this trend and suggest that life spans may be even shorter in tropical species.

In summary, the size-at-age data for tropical squids suggest a demographic model characterised by a rapid linear or exponential growth pattern and short life span. Under these circumstances, reproduction would occur concurrently with rapid somatic growth. The data also suggest more rapid growth rates and shorter life spans in tropical species relative to temperate species, perhaps as a function of temperature.

Acknowledgments

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Development of Hypoosmoregulatory Capacity in Arctic char (*Salvelinus alpinus*) Reared under either Continuous Light or Natural Photoperiod

Arne M. Arnesen

Norwegian Institute of Fisheries and Aquaculture, P.O. Box 677, N-9001 Tromsø, Norway

Morten Halvorsen

Troms County Environmental Protection Department, P.O. Box 595, N-9001 Tromsø, Norway

and Kjell J. Nilssen

Department of Zoology, University of Trondheim, AVH, N-7055 Dragvoll, Norway

Arnesen, A. M., M. Halvorsen, and K. J. Nilssen. 1992. Development of hypoosmoregulatory capacity in Arctic char (*Salvelinus alpinus*) reared under either continuous light or natural photoperiod. Can. J. Fish. Aquat. Sci. 49: 229–237.

Two groups of Arctic char (*Salvelinus alpinus*) were reared in freshwater (5-6°C) under either continuous light (LDL) from first feeding (March) or LDL until July and then natural photoperiod (NDL, 70°N). Direct transfer to seawater (5.5°C, 35 ppt) in February resulted in both groups exhibiting increases in blood plasma osmolality, Na^+, and Mg^{2+} concentrations and a significant decrease in muscle water content. When tested in May, an improvement in seawater tolerance was evident in both groups. In June, only the NDL fish showed further improvements in hypoosmoregulatory capacity, since they exhibited only minor fluctuations in plasma constituents and muscle water content following direct transfer to seawater. Increased body size could partially explain the improved seawater tolerance in the experimental groups. Acclimation to brackish water prior to transfer to 35 ppt seawater in June improved seawater tolerance only in fish reared under continuous light. The results indicate that the seasonal increase in photoperiod stimulates the development of hypoosmoregulatory capacity whilst the fish are still resident in freshwater.

On a élevé deux groupes d'omble chevalier (*Salvelinus alpinus*) en eau douce de 5 à 6°C dans des conditions d'éclairement continu à partir du début de l'alimentation (mars), et dans ces mêmes conditions jusqu'en juillet, pour ensuite les soumettre à la photopériode naturelle retrouvée à 70° de latitude nord. Le transfert en eau salée (5,5°C, 35 parties par mille (ppm)) en février a entraîne une augmentation de l'osmolalité du plasma sanguin et des concentrations de Na^+ et de Mg^{+2}, ainsi qu'une baisse nette de la teneur en eau des muscles chez les deux groupes. Lorsque ceux-ci ont été examinés en mai, ils ont tous deux montré une amélioration de leur tolérance à l'eau de mer. En juin, seul le groupe exposé à une photopériode naturelle avait encore amélioré sa capacité hypoosmorégulatoire; ainsi, il ne montrait que de légères fluctuations des éléments du plasma et des teneurs en eau des muscles après son transfert en eau salée. L'augmentation de la taille peut en partie expliquer la tolérance améliorée à l'eau salée des groupes expérimentaux. L'acclimatation à l'eau saumâtre avant le transfert en eau salée de 35 ppm en juin n'a entraîné une amélioration de la tolérance à l'eau salée que chez le groupe élevé dans des conditions d'éclairement continu. Les résultats obtenus portent à croire que l'augmentation saisonnière de la photopériode stimule le développement de la capacité hypoosmorégulatoire pendant que les poissons vivent encore en eau douce.

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In northern Norway, anadromous Arctic char (*Salvelinus alpinus*) may perform one to three sea migrations before first spawning (Nordeng 1983). These migrations are limited to a period of 4–6 wk during the summer season (Mathisen and Berg 1968). In contrast, most of the *Salmo* and *Oncorhynchus* species usually make a single seaward migration during their lifetime. These species undergo a parr–smolt transformation (smoltification) which preadapts them to a marine life (McCormick and Saunders 1987; Hoar 1988). The most important environmental factor modulating the process is photoperiod (McCormick and Saunders 1987; Hoar 1988), but hypoosmoregulatory ability is also strongly influenced by body size (Parry 1958; Jackson 1981; McCormick and Naiman 1984b; McCormick and Saunders 1987; Hoar 1988). Little, however, is known about the smoltification process and the development of seawater tolerance in Arctic char.

Arctic char held in brackish water at ambient temperature and reared under natural photoperiod showed a seasonal change in hypoosmoregulatory capacity (Finstad et al. 1989). These results would indicate both the existence of an anticipatory development of seawater tolerance in the Arctic char and that environmental factors might influence the timing of these changes. The findings of Finstad et al. (1989) are of additional interest, since it has been suggested that the development of

seawater tolerance in anadromous brook trout (*Salvelinus fontinalis*) depends upon estuarine residence (McCormick et al. 1985).

McCormick and Saunders (1987) suggested that preparatory physiological adaptions for seawater entry would not occur in species that make repeated seawater entries, but would only occur in species that make a single seaward migration during their lifetime. This study was designed in order to test this hypothesis. The existence of anticipatory hypoosmoregulatory development and the potential influence of photoperiod on the development of seawater tolerance were examined. The seawater tolerance of Arctic char reared in freshwater under conditions of constant temperature and either natural photoperiod or constant light was tested. We have also examined the effect of a brackish water acclimation period before seawater exposure. In addition, tests were performed to quantify the relationship between body size and hypoosmoregulatory performance in our experimental fish.

Material and Methods

Experimental Animals and Rearing Conditions

The Arctic char (derived from the anadromous strain of Lake Storvatn, Hammerfest, 70°30′N) were obtained as eyed eggs from a hatchery (A/S Sjøblink) in January 1987. Eggs were incubated under ambient temperature conditions (1–1.5°C). The eggs hatched in February and the alevins were first fed at the end of March. From first feeding, alevins were kept under continuous light and at a water temperature of 5–6°C.

At a size of about 2 g (July 1987), the char were transferred to rearing tanks covered either with transparent polyester (outdoors) or with a black, nontransparent polythene sheet (indoors). Group A ($n = 1000$) was exposed to natural outdoor photoperiod (NDL, natural daylength) and group B ($n = 1000$) to continuous illumination (LDL, long daylength) from a white bulb (Osram, 40 W) placed 40 cm above the water surface. A third group (C) of fish (approximately 5000 individuals) was distributed amongst several rearing tanks in the hatchery and reared under the same conditions as group B except that the tanks were not covered with a black polythene sheet.

The rearing tanks (1.5 × 1.5 m) had a water depth of 40 cm and a volume of approximately 900 L. Water flow was increased from 5 L/min (July 1987) to 15 L/min (May 1988) to compensate for the increasing biomass in the tanks. The fish were continuously fed commercial dry pellet feed (Ewos, ST 42) using automatic disc feeders. Pellet size was adjusted to fish size. The amount of food to be given was estimated from fish size and water temperature according to a growth model for Arctic char (Jobling 1983) and assuming a conversion ratio of 1:1. Freshwater temperature was kept at 5.5°C (±1°C) throughout the experimental period (September 1987 to June 1988).

Seawater Tolerance

Seawater tolerance will be defined as the ability to survive and hypoosmoregulate in seawater (>30 ppt salinity). Hypoosmoregulatory ability was assessed by exposing 45 fish, sampled at random from each experimental group, to natural seawater (35 ppt) at the rearing temperature (5.5 ± 0.5°C) for 7 d. The fish were starved for 2 d before the start of the seawater exposure test. Only immature fish were used in the trials. The fish were transferred directly from the rearing tanks to test tanks (one tank for each experimental group) containing 500 L of seawater. The water in the test tanks was aerated using pumps (Rena 301) and passed through charcoal filters using recirculation pumps (Eheim 1017). In order to prevent accumulation of nitrogenous waste products, 50% of the seawater was changed each day. Seawater concentration was measured by use of a salinity refractometer (Atago). Osmotic and ionic concentrations in seawater of different salinities are given in Barnes (1954). The challenge tests were performed under conditions of continuous light, but with test tanks covered to reduce the light intensity.

Sampling Procedure and Analyses

Blood and muscle tissue were sampled from 8–10 fish in each test group at the following times: before transfer to test tanks with seawater (day 0) and after 1, 2, 3, 5, and 7 d in seawater (days 1–7). Fish sampled from the test tanks were killed by a blow to the head and blood was collected (within 60 s) from the caudal vessels with a syringe, transferred to a heparinized plastic tube, and centrifuged (5 min, 5500 rpm). Thereafter, plasma was removed and stored at −20°C until analysed for osmolality (Wescor 5500), Na^+ (Radiometer FLM 3), and Mg^{2+} (Perkin-Elmer 300). Muscle tissue (1–2 g) was taken from the region below the dorsal fin, weighed to the nearest 0.01 g, packed in aluminium foil, and frozen (−20°C) until analysed for water content (determined as loss of weight after 24 h of drying at 105°C). Body weight (nearest gram) and total length (nearest millimetre) were measured before muscle sampling. All sampled fish were dissected, sex determined, and immature status confirmed. Mortality within test groups was monitored each day.

Seasonal Changes

In order to investigate seasonal differences in the ability of Arctic char to perform hypoosmotic regulation, seawater tolerance tests were carried out with fish from groups A and B on three different occasions in 1988: from 24 February to 2 March, from 9 to 16 May, and from 6 to 13 June. These periods will hereafter be referred to as February, May, and June, respectively. The test fish had a mean total length (±SD) of 18.6 ± 1.7 (LDL) and 16.9 ± 1.1 cm (NDL), 20.7 ± 1.5 (LDL) and 20.1 ± 1.6 cm (NDL), and 21.8 ± 1.9 (LDL) and 21.4 ± 1.8 (NDL) cm in February, May, and June, respectively.

Acclimation to Brackish Water

To examine the effect of brackish water acclimation on subsequent performance in the seawater trials, 55 fish each from groups A and B were kept in dilute seawater (15–17 ppt salinity, 5–7°C) for a period of 3 wk prior to the June seawater tolerance test.

Effects of Body Size

To quantify the relationship between body size and hypoosmoregulatory performance, seawater tolerances of five different size groups of Arctic char were tested in late March (17–24) 1988. Fish from group C were carefully size graded by hand into five different length groups (LG): LG 1, 135–152 mm (146 ± 3.8); LG 2, 154–166 mm (160 ± 3.2); LG 3, 167–178 mm (172 ± 2.9); LG 4, 180–188 mm (183 ± 2.6); LG 5, 206–232 mm (216 ± 6.6). The seawater tolerance tests were performed as described above.

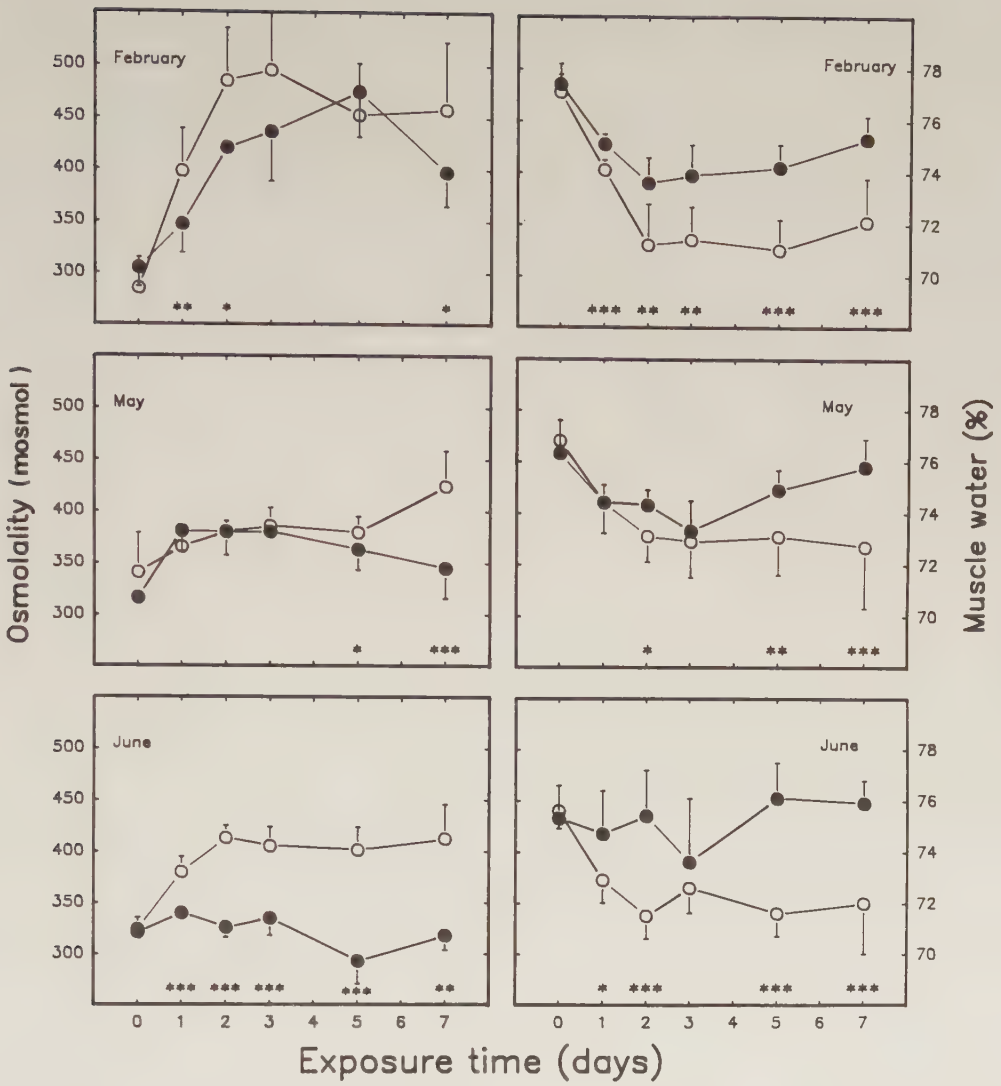

FIG. 1. Blood plasma osmolality and muscle water content in Arctic char exposed to seawater (35 ppt, 5.5°C) during February, May, and June. Values on day 0 are for control fish held in freshwater. Filled circles represent fish reared under natural photoperiod and open circles represent fish reared under continuous light. Points represent means ± SD. Asterisks indicate significant differences between the two groups ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$).

Statistical Analyses

All values reported are means (± 1 SD). Interseasonal differences between experimental groups were assessed by the Mann–Whitney *U* test, and the Kruskal–Wallis test was used to evaluate differences among the different groups with respect to time. The Kruskal–Wallis test or ANOVA was used to test differences among the different months; a posteriori Tukey–Kramer tests were used to identify where significant differences occurred among the class levels. When performing Tukey–Kramer tests, log-transformed data were used when appropriate. Linear regression was used to assess the relationship between the measured physiological parameters and fish size. A probability level of less than 0.05 was considered significant in all tests.

Results

Seawater Challenge Tests in February

When they were exposed to seawater in February, no mortality occurred in fish that had been reared under NDL. Four fish of a total of 45 from the LDL group died. Plasma osmolality increased significantly in both groups (Fig. 1) to maximum levels of 495 mosmol on day 3 (LDL) and 475 mosmol on day 5 (NDL). At three of the sampling times (days 1, 2, and 7), plasma osmolalities were significantly lower in NDL fish than in LDL fish (Fig. 1). In both groups, there were significant increases in plasma concentrations of Na^+ and Mg^{2+} (Fig. 2) and a significant reduction of muscle water content (Fig. 1). Muscle water content and plasma Na^+ levels differed significantly between the two groups of fish (NDL and LDL), whilst plasma levels of Mg^{2+} did not.

Seawater Challenge Tests in May

Although plasma osmolalities increased in both groups during seawater challenge tests in May (Fig. 1), levels were not as high as in February, and no mortalities occurred. In NDL fish, plasma osmolality increased from a freshwater level of 316 to 381 mosmol after 1 d in seawater, but had fallen to 345 mosmol within 7 d. Plasma osmolality increased with

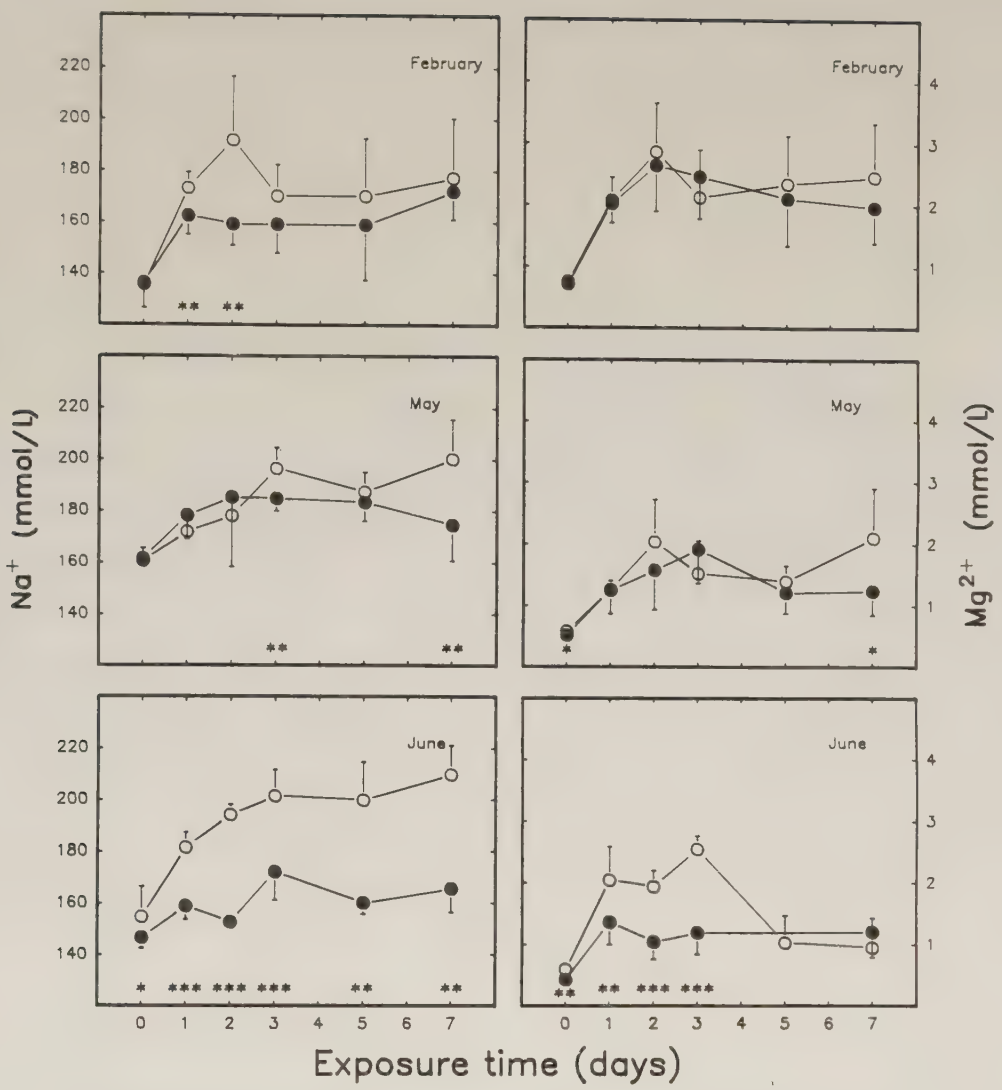

FIG. 2. Blood plasma Na^+ and Mg^{2+} concentrations in Arctic char exposed to seawater at different times of the year. Experimental conditions and symbols as in Fig. 1.

exposure time in LDL fish, reaching 424 mosmol by the end of the test. Significant differences in plasma osmolality were found between the two groups on days 5 and 7.

Reduced muscle water content was observed in both groups following transfer to seawater (Fig. 1). With the exception of the NDL group fish on day 7, values of muscle water content were significantly lower following seawater exposure than freshwater values (day 0). Thus, seawater exposure resulted in dehydration of muscle tissue. Changes in plasma Na^+ concentrations appeared to follow a similar pattern to the changes in plasma osmolality (Fig. 2). Mean plasma Mg^{2+} values differed significantly between the two groups on day 0 (freshwater) and day 7 (Fig. 2).

Seawater Challenge Tests in June

There were large differences in hypoosmoregulatory capacity between the two groups of fish when they were exposed to seawater in June. No mortality occurred in NDL fish, whilst 7 of 45 LDL fish died during the test. In NDL fish, there were only minor changes in plasma osmolality following transfer to seawater (Fig. 1). By contrast, plasma osmolality in LDL fish had increased to 413 mosmol by day 2 after seawater transfer and stabilized at this level thereafter. The plasma osmolality levels of LDL fish were significantly higher than those of NDL fish throughout the entire exposure period.

There were also differences between the NDL and LDL fish in muscle water content (Fig. 1) and in plasma concentrations of Na^+ and Mg^{2+} (Fig. 2). In general, these parameters reflected the same differences in hypoosmoregulatory capacity between NDL and LDL fish as did plasma osmolality levels.

Seasonal Differences in Hypoosmoregulatory Capacity

In NDL fish the hypoosmoregulatory capacity improved during the experimental period from February to June. The results presented in Table 1 indicate at which sampling times significant differences were observed in the experiments carried out during the different months. There was a general trend for plasma osmolality to be lower in June than in the other two months during which experiments were conducted. Values of plasma osmolality recorded in May tended to be lower than those recorded in February. Differences in plasma concentrations of Mg^{2+} followed the same pattern as plasma osmolality,

TABLE 1. Seasonal differences in seawater tolerance in Arctic char reared in freshwater under natural light conditions (NDL). The results summarize statistical analyses (Tukey–Kramer tests) of differences in physiological parameters between months (mean values), measured at different times following exposure to seawater. The original data are presented in Fig. 1–4. Symbols indicate whether the values recorded during sampling in the first month are significantly higher (+) or lower (−) than those of the second month (+/− = $p < 0.05$, ++/−− = $p < 0.01$, +++/−−− = $p < 0.001$), n.s. = nonsignificant. Day 0 represents values from control fish held in freshwater.

	Exposure time					
Parameter	Day 0	Day 1	Day 2	Day 3	Day 5	Day 7
Plasma osmolality						
February vs. May	n.s.	−	++	+++	+++	++
May vs. June	n.s.	++	++	+	++	n.s.
February vs. June	−	n.s.	+++	+++	+++	+++
Muscle water						
February vs. May	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
May vs. June	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
February vs. June	++	n.s.	−	n.s.	−−	n.s.
Plasma Na^+						
February vs. May	−−−	−−	−	−−−	−−	n.s.
May vs. June	+++	+++	++	+	+	n.s.
February vs. June	−	n.s.	n.s.	−	n.s.	n.s.
Plasma Mg^{2+}						
February vs. May	+++	++	++	n.s.	+	+
May vs. June	+	n.s.	n.s.	+		n.s.
February vs. June	+++	+++	+++	++		+

TABLE 2. Seasonal differences in seawater tolerance in Arctic char reared in freshwater under conditions of continuous light (LDL). Analyses, presentation, and symbols as in Table 1.

	Exposure time					
Parameter	Day 0	Day 1	Day 2	Day 3	Day 5	Day 7
Plasma osmolality						
February vs. May	−−	n.s.	+++	++	++	n.s.
May vs. June	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
February vs. June	−	n.s.	+++	++	+	n.s.
Muscle water						
February vs. May	n.s.	n.s.	−−	n.s.	−	n.s.
May vs. June	++	++	+	n.s.	n.s.	n.s.
February vs. June	+++	+	n.s.	n.s.	n.s.	n.s.
Plasma Na^+						
February vs. May	−−−	n.s.	n.s.	−−−	n.s.	−
May vs. June	n.s.	−	n.s.	n.s.	n.s.	n.s.
February vs. June	−−−	n.s.	n.s.	−−−	−−	−−
Plasma Mg^{2+}						
February vs. May	++	++	+	n.s.	+	n.s.
May vs. June	n.s.	−−	n.s.	−−	n.s.	++
February vs. June	++	n.s.	+	n.s.	++	+++

but examination of plasma Na^+ concentrations revealed no clear-cut trends. Muscle water content was generally lower following seawater exposure in February than in June (Fig. 1; Table 1), indicating that muscle dehydration was greater in February than in June.

The LDL fish exhibited improved hypoosmoregulatory capacity from February to May, but no further improvement was found when the fish were exposed to seawater in June (Table 2).

Seawater Challenge Tests of Brackish Water Acclimated Fish

All fish survived both the acclimation period in brackish water and subsequent exposure to seawater in June. In NDL fish, no increases in plasma osmolality were seen in the period following exposure to seawater (Fig. 3). LDL fish displayed increasing levels of plasma osmolality throughout the test period, with most values being significantly higher than the values recorded for NDL fish acclimated to brackish water. For the LDL fish acclimated to brackish water, all the plasma osmolality values were significantly lower than values recorded for the LDL fish transferred directly from freshwater to seawater.

Muscle water content and plasma levels of Na^+ and Mg^{2+} also differed between the brackish water acclimated NDL and LDL groups (Fig. 3). In NDL fish, no significant changes

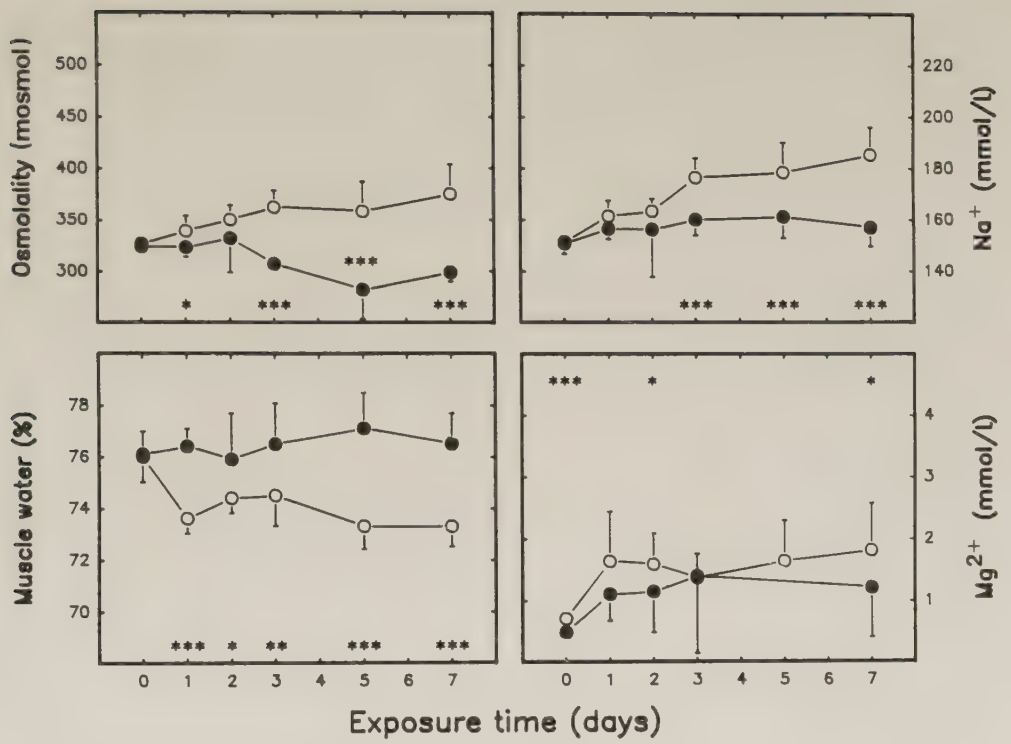

FIG. 3. Blood plasma osmolality, Na^+ and Mg^{2+} concentrations, and muscle water content in Arctic char exposed to seawater in June following 21 d of acclimation to brackish water (15–17 ppt salinity). Symbols as in Fig. 1.

occurred in muscle water content or plasma Na^+ concentrations after transfer to seawater.

Effect of Body Size on Hypoosmotic Performance

In fish sampled from freshwater immediately prior to seawater transfer, there were significant positive linear relationships between body length and plasma osmolality and plasma concentrations of Na^+ and Mg^{2+} (Table 3). There was also found to be a significant negative linear relationship between body length and muscle water content.

During the course of 7 d of seawater exposure the only mortalities occurred in LG 1 (three fish), LG 2 (four fish), and LG 4 (one fish). After seawater transfer, plasma osmolality increased from 297 to 325 mosmol in freshwater, to reach maxima of 441–475 mosmol on day 5, in all groups (Table 4). A significant negative linear relationship was found between body length and plasma osmolality on days 2, 3, 5, and 7 (Table 3), indicating differences in hypoosmotic regulatory performance between the different size groups.

An inverse relationship was seen between plasma osmolality and muscle water content. Muscle water content decreased to minimum levels on day 3 or 5 (Table 4). There was a significant positive linear relationship between body length and muscle water content on day 7 (Table 3).

In general, plasma Na^+ levels showed the same trends as changes in plasma osmolality. During seawater exposure, maximum levels were reached on day 3 or 5 (Table 4). There was a significant negative linear relationship between body length and plasma Na^+ concentration on days 2, 5, and 7 (Table 3).

After transfer to seawater, plasma Mg^{2+} concentrations reached maximum values on day 5 in LG 3, LG 4, and LG 5 and on day 7 in LG 1 and LG 2 (Table 4). Thus, Mg^{2+} levels seemed to increase steadily during the course of the entire test period in the smallest fish. A significant negative relationship was found between body length and plasma Mg^{2+} concentration on days 2 and 7 (Table 3).

Discussion

McCormick and Saunders (1987) hypothesized that preparatory physiological adaptations for seawater entry would not occur in species that make repeated seawater entries, but would only occur in species that make a single seaward migration during their lifetime. The results of our experiments seriously question the validity of this hypothesis.

Results from the seawater challenge tests carried out on the NDL group demonstrate that the fish had developed improved hypoosmoregulatory capacity between February and May during the period of increasing daylength. When tests were carried out in June (at maximal NDL = 24 h of light), blood plasma osmolality did not exceed 340 mosmol and the increase over levels recorded in freshwater fish was transitory. Following 7 d of seawater exposure, blood plasma osmolality in the NDL fish was slightly lower than the initial freshwater level. Thus, at the time when free-living anadromous Arctic char would be migrating towards the sea, our fish exhibited seawater tolerance comparable with that observed in functional salmon smolts (Clarke and Blackburn 1977). These results confirm the findings of Finstad et al. (1989) who demonstrated seasonal differences in seawater tolerance in Arctic char reared in brackish water. In addition, we have established that Arctic char, like salmonids undergoing the parr–smolt transformation, anticipate seaward migration by developing hypoosmoregulatory capacity whilst

TABLE 3. Effects of body length (mm) on plasma osmolality (Osm), muscle water content (Mw), and plasma Na^+ and Mg^{2+} concentrations in Arctic char kept in freshwater (day 0) or exposed to seawater for 1–7 d. Slope (*a*), *y*-intercept (*y*-int.), sample size (*n*), coefficient of determination (r^2), and significance of regression slope (*p*; t-test) are indicated. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, n.s. = nonsignificant.

Exposure time	Parameter			
	Osm (mosmol)	Mw (%)	Na^+ (mmol/L)	Mg^{2+} (mmol/L)
Day 0				
a	0.32	−0.01	0.16	0.002
y-int.	252.4	79.6	113.6	0.20
n	50	50	50	50
r^2	0.36	0.14	0.26	0.39
p	***	**	***	***
Day 1				
a	0.02	−0.01	0.00	−0.009
y-int.	363.1	76.6	168.4	3.74
n	50	49	50	50
r^2	0.00	0.07	0.00	0.07
p	n.s.	n.s.	n.s.	n.s.
Day 2				
a	−0.67	0.01	−0.19	−0.014
y-int.	509.0	71.5	214.0	5.04
n	52	52	51	52
r^2	0.39	0.06	0.19	0.17
p	***	n.s.	***	**
Day 3				
a	−0.48	0.01	0.05	−0.005
y-int.	462.3	71.5	174.1	3.53
n	50	50	49	50
r^2	0.09	0.01	0.01	0.01
p	*	n.s.	n.s.	n.s.
Day 5				
a	−0.48	0.00	−0.13	−0.010
y-int.	543.3	72.7	212.1	4.77
n	48	48	48	48
r^2	0.22	0.00	0.09	0.04
p	***	n.s.	*	n.s.
Day 7				
a	−0.84	0.02	−0.20	−0.032
y-int.	571.5	69.9	211.1	8.59
n	39	44	39	39
r^2	0.40	0.15	0.19	0.25
p	***	**	**	***

still residing in freshwater. The results are further supported by the findings of a high hypoosmoregulatory capacity in descending, anadromous Arctic char from a natural population (M. Halvorsen, Troms County Environmental Protection Department, P.O. Box 595, N-9001 Tromsø, Norway, pers. comm.).

Since only small changes in plasma Na^+ and Mg^{2+} concentrations occurred in NDL fish during seawater exposure in June, the hypoosmoregulatory capacity in the char was probably related to a high activity of the gill-bound Na^+,K^+-ATPase system, as demonstrated by Finstad et al. (1989), and to a well-developed Mg^{2+} regulatory function in the kidneys.

The only environmental factor that was varied for the NDL group was photoperiod. It is therefore likely that changes in photoperiod provided the zeitgeber for the osmoregulatory developments. This conclusion appears to be supported by the results obtained from the group of fish reared under continuous light (LDL). These char failed to perform the same osmoregulatory changes as seen in the NDL group. Photoperiodic cues have previously been demonstrated to be essential for the development of hypoosmoregulatory capacity in species such as Atlantic salmon (*Salmo salar*) (Saunders and Henderson 1970; Komourdjian et al. 1976; Clarke et al. 1985; Saunders et al. 1985; McCormick et al. 1987), steelhead trout (*Oncorhynchus mykiss*) (Wagner 1974), and sockeye (*O. nerka*) and coho salmon (*O. kisutch*) (Clarke et al. 1978). In teleost fish, photoperiod probably acts as a zeitgeber through the light–melatonin system (Falcon et al. 1989). By contrast, McCormick and Naiman (1984b) reported that body size, not photoperiod, was the determining factor for the development of seawater tolerance in brook trout, another species within the *Salvelinus* genus. In the two different forms of *O. mykiss*, rainbow trout and steelhead trout, it seems that photoperiod-stimulated development of seawater tolerance occurs only in the latter form (Johnsson and Clarke 1988).

In our experiments with fish from different length groups, we found positive linear relationships between body length and plasma osmolality and Na^+ and Mg^{2+} concentrations of Arctic char kept in freshwater. A negative linear relationship was found between length and muscle water content. Thus, body size apparently affects the regulation of plasma ions in Arctic char kept in freshwater. This is in accordance with earlier reports for brook trout (McCormick and Naiman 1984a).

When different size groups of Arctic char were exposed to seawater, the highest mortalities, and the highest levels of plasma ions and osmolality, were found in the smallest length groups. Negative linear relationships were found between body length and plasma osmolality and Na^+ and Mg^{2+} concentrations. A positive linear relationship was found between body length and muscle water content. These results indicate that body size affects hypoosmoregulatory performance, and seawater tolerance, in Arctic char. This seems to be a general phenomenon in salmonids (Parry 1958; Jackson 1981; McCormick and Naiman 1984b; McCormick and Saunders 1987; Hoar 1988). Size-dependent seawater tolerance may be caused by a more favorable area to volume ratio in big fish and/or a gradually increasing osmoregulatory capacity with size (McCormick and Saunders 1987).

Plasma osmolality values recorded when Arctic char of about 216 mm length (LG 5) were exposed to seawater in March were considerably higher than recorded for NDL fish exposed to seawater in June (Table 4; Fig. 1). Since these two groups of fish were of approximately the same size, an increase in body size can probably only partially account for the observed improvement in hypoosmoregulatory capacity in NDL fish during the experimental period from February to June.

Despite being maintained under conditions of continuous light, the LDL group also exhibited improved salinity tolerance from February to May. There was, however, no improvement in seawater tolerance in this group from May to June. These changes may be the result of the existence of an endogenous circannual rhythm controlling the parr–smolt transformation, as has been suggested for salmonids by other authors (Hoar 1965, 1988; Wagner 1974; Eriksson and Lundqvist 1982; Duston and Saunders 1990).

Gradual acclimation to increasing salinities has been reported to result in improved seawater survival in resident Arctic char by Roberts (1971) and in anadromous brook trout by McCormick and Naiman (1984b). Three weeks of acclimation

TABLE 4. Plasma osmolality (Osm), muscle water content (Mw), and plasma Na^+ and Mg^{2+} concentrations in Arctic char from different size groups exposed to seawater (35 ppt, 5.5°C) for 7 d. Values indicate means (SD). Day 0 represents values from control fish held in freshwater. Sample sizes were between 8 and 11.

Exposure time (d)	Length groups (mm) 1 (135–152)		2 (154–166)		3 (167–178)		4 (180–188)		5 (206–232)	
Osm (mosmol)										
0	306	(10.3)	297	(9.3)	313	(7.3)	307	(10.4)	325	(8.3)
1	367	(26.8)	356	(21.3)	363	(15.9)	382	(18.0)	361	(26.3)
2	418	(38.0)	397	(17.3)	392	(16.1)	383	(15.6)	369	(11.2)
3	418	(37.7)	398	(17.2)	404	(20.4)	423	(31.9)	386	(15.4)
5	475	(22.6)	468	(21.9)	461	(19.5)	454	(26.3)	441	(12.0)
7	453	(29.6)	435	(28.2)	429	(21.4)	411	(17.7)	395	(33.6)
Mw(%)										
0	77.7	(0.7)	77.3	(1.0)	77.8	(0.6)	77.8	(0.3)	76.8	(0.6)
1	74.4	(0.7)	74.4	(0.6)	74.8	(1.0)	73.2	(2.1)	73.6	(1.1)
2	72.7	(1.0)	73.1	(0.8)	73.3	(0.9)	73.3	(0.9)	73.5	(0.9)
3	71.9	(1.1)	73.1	(1.1)	72.9	(0.9)	71.9	(1.5)	72.8	(1.0)
5	72.6	(1.2)	72.0	(0.9)	72.4	(0.8)	72.4	(1.4)	72.4	(0.8)
7	73.6	(1.5)	73.3	(1.3)	73.6	(1.1)	74.2	(1.5)	74.6	(1.3)
Na^+ (mmol/L)										
0	141	(5.5)	136	(8.7)	144	(4.0)	139	(4.7)	150	(5.3)
1	167	(11.6)	164	(12.4)	170	(6.7)	174	(10.7)	164	(8.2)
2	187	(16.5)	184	(8.1)	182	(5.7)	176	(8.7)	175	(6.7)
3	181	(16.3)	174	(18.1)	180	(11.8)	194	(17.2)	179	(11.2)
5	194	(12.8)	191	(11.3)	190	(7.6)	186	(11.1)	185	(5.2)
7	185	(7.1)	175	(8.4)	180	(8.4)	171	(12.5)	170	(10.5)
Mg^{2+} (mmol/L)										
0	0.49	(0.04)	0.46	(0.05)	0.55	(0.05)	0.52	(0.07)	0.61	(0.05)
1	2.47	(0.74)	2.26	(0.57)	2.27	(0.69)	2.45	(1.09)	1.73	(0.46)
2	3.06	(1.18)	2.74	(0.67)	2.71	(0.70)	2.44	(0.40)	2.14	(0.70)
3	3.07	(1.49)	2.28	(0.65)	2.61	(1.09)	3.28	(2.12)	2.20	(1.36)
5	3.40	(1.25)	3.02	(0.52)	3.06	(1.10)	3.19	(1.60)	2.46	(0.92)
7	3.83	(0.93)	3.65	(2.21)	2.61	(0.60)	2.89	(1.10)	1.71	(1.28)

to brackish water (15–17 ppt salinity) prior to seawater exposure in June improved hypoosmoregulatory ability in LDL fish, but had little or no effect in NDL fish. These results are in accord with the findings that freshwater-reared NDL fish displayed excellent hypoosmoregulatory capacity at this time of the year. Acclimation to brackish water does not seem to be necessary for the establishment of full seawater tolerance in Arctic char that have experienced appropriate changes in photoperiod. Brackish water acclimation may, however, lead to improved hypoosmoregulatory capacity in fish that are not physiologically prepared for entering highly saline waters.

The development of hypoosmoregulatory mechanisms in anadromous Arctic char, which have annual migrations between freshwater and the sea, is preparatory and does not depend upon estuarine residence. In agreement with this, Johnson and Heifetz (1988) reported that descending, anadromous Dolly Varden char (*Salvelinus malma*) had well-developed hypoosmoregulatory capacity. Thus, at least two *Salvelinus* species seem to have developed preparatory physiological adaptations for a marine life.

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Distribution of Early-Stage Atlantic Cod (*Gadus morhua*), Haddock (*Melanogrammus aeglefinus*), and Witch Flounder (*Glyptocephalus cynoglossus*) Eggs on the Scotian Shelf: A Reappraisal of Evidence on the Coupling of Cod Spawning and Plankton Production

Keith Brander[1] and Peter C. F. Hurley

Department of Fisheries and Oceans, Marine Fish Division, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, N.S. B2Y 4A2, Canada

Brander, K., and P. C. F. Hurley. 1992. Distribution of early-stage Atlantic cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), and witch flounder (*Glyptocephalus cynoglossus*) eggs on the Scotian Shelf: a reappraisal of evidence on the coupling of cod spawning and plankton production. Can. J. Fish. Aquat. Sci. 49: 238–251.

In spring, Atlantic cod (*Gadus morhua*) spawned progressively later from southwest to northeast along the Scotian Shelf and matched the variation in peak abundance of *Calanus finmarchicus*. In fall, cod spawned in some, but not all, areas where *Calanus* were abundant. This is consistent with the hypothesis that cod spawning is coupled to copepod production, which is a part of the "match–mismatch" hypothesis proposed by D. H. Cushing. The timing of spawning was estimated from the distributions of early- and late-stage eggs of cod, haddock (*Melanogrammus aeglefinus*), and witch flounder (*Glyptocephalus cynoglossus*) collected during Scotian Shelf Ichthyoplankton Survey (SSIP) cruises in 1979–81.

Au printemps, la morue *Gadus morhua* a frayé de plus en plus tard suivant un axe sud-ouest/nord-est le long de la plate-forme Scotian. La fraie a coincidé avec la variation de l'abondance de pointe de *Calanus finmarchicus*. À l'automne, la morue a frayé dans certaines régions d'abondance où les *Calanus* étaient abondants, mais pas dans toutes. Ceci concorde avec l'hypothèse selon laquelle la fraie de la morue est liée à la production de copépodes, hypothèse qui s'inscrit dans l'hypothèse "pair–impair" de D. H. Cushing. Le moment de la fraie a été déterminé à partir de la répartition des oeufs du premier stade et du stade avancé de morue, d'aiglefin (*Melanogrammus aeglefinus*) et de plie grise (*Glyptocephalus cynoglossus*) recueillis au cours des expéditions de relevés de l'ichtyoplancton de la plate-forme Scotian effectuées de 1979 à 1981.

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The Scotian Shelf Ichthyoplankton Programme (SSIP) of the Canadian Department of Fisheries and Oceans was designed to provide basic information on the spatial and temporal distribution of eggs and larvae on the Scotian Shelf. Several studies have used the SSIP data set to look at temporal and spatial distribution of ichthyoplankton communities (O'Boyle et al. 1984), timing and location of cod spawning (Gagné and O'Boyle 1984), and stock structure of flatfish (Neilson et al. 1988). However, in the case of Atlantic cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), and witch flounder (*Glyptocephalus cynoglossus*), which can only be distinguished from each other when the embryo extends almost all the way round the egg, these studies only used information on late-stage eggs. Early-stage eggs provide more information on location and timing of spawning because they are an order of magnitude more numerous and are closer to the position of spawning. The average age of stage 1–3 cod eggs (at average temperature 3–4°C and average instantaneous egg mortality rate of $0.15 \cdot d^{-1}$) is about 7 d, compared with about 20 d for a stage 4 egg (Thompson and Riley 1981; but see Markle and Frost 1985 for egg stages used here). Species proportions of early-stage eggs of the three species can be estimated from proportions of older, identified eggs using methods similar to those used for estimating proportions at age in an age–length key (Brander 1974).

We reexamine the location and timing of spawning of cod, haddock, and witch flounder on the Scotian Shelf, using information on the distribution of early-stage eggs. Cod spawning is discussed in relation to seasonal cycles of temperature and plankton and compared with previous conclusions reached for this area. Gagné and O'Boyle (1984), for example, observed that cod on the Scotian Shelf spawn over a long period of the year and that "A match between the seasonal blooms and the time of spawning does not appear to be essential, although it might exist in the spring." This apparently contradicts the hypothesis that the timing of spawning is related to the timing of plankton production (or specifically, that cod spawning is related to *Calanus* production; Cushing 1984). We shall refer to this as the "match" hypothesis, since it is a necessary part of the "match–mismatch" hypothesis (Cushing 1969). The "match–mismatch" hypothesis can be divided into two parts: (a) timing of spawning is coupled to timing of plankton production and (b) variability in timing of plankton production leads to variability in larval mortality and hence in year class strength. This paper is only concerned with the first part of the "match–mismatch" hypothesis and not with variability in year class strength.

[1]Current address: Ministry of Agriculture, Fisheries and Food, Fisheries Laboratory, Lowestoft, Suffolk NR33 0HT, United Kingdom.

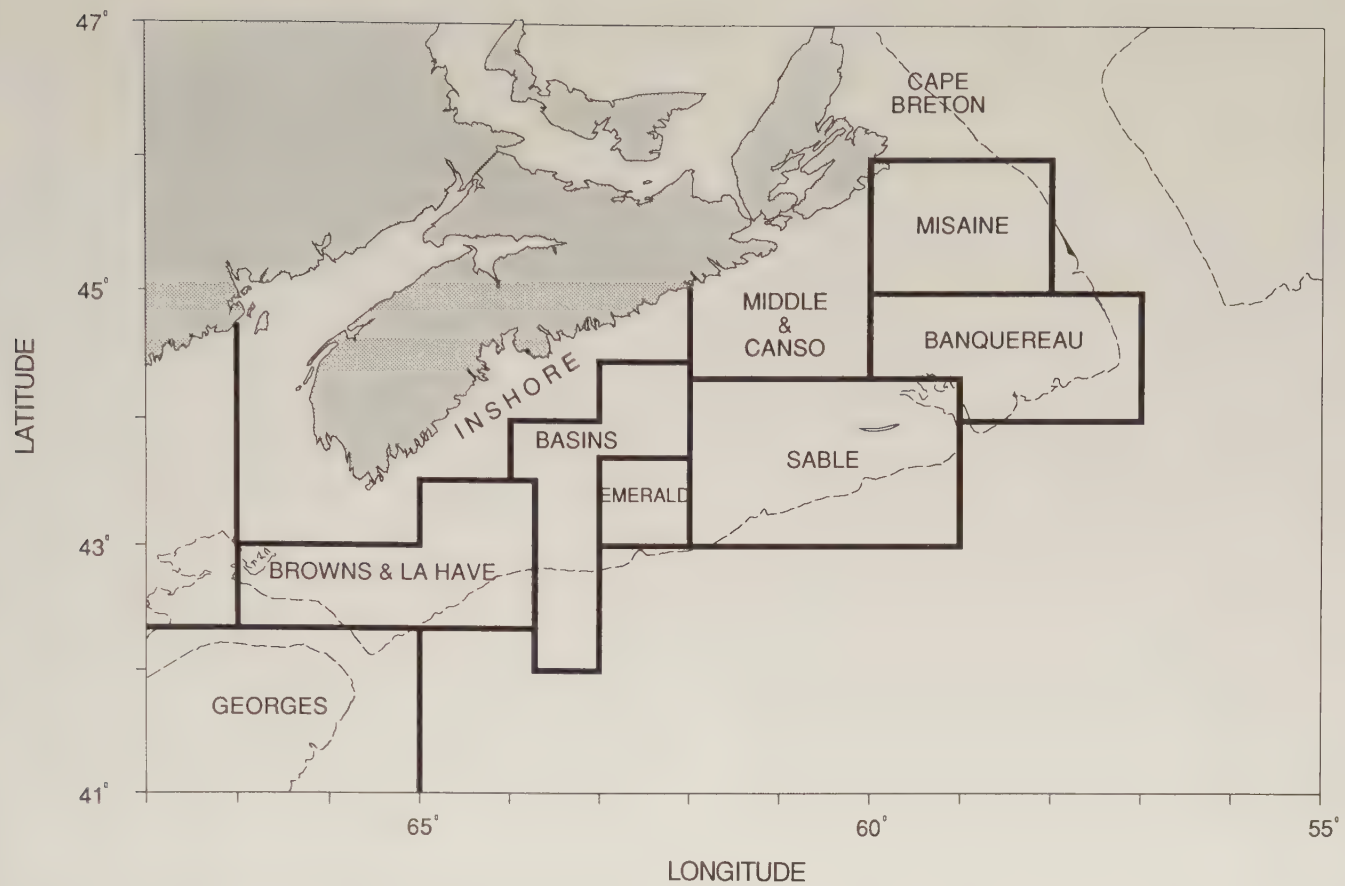

FIG. 1. Subareas of the Scotian Shelf used to estimate species composition of early-stage eggs of cod, haddock, and witch flounder.

TABLE 1. Cruise by cruise summary of numbers of cod, witch flounder, and haddock eggs per square metre and mean surface temperature, Cape Breton area.

Cruise	Number of stations	Day of year	Year	Identified (stage 4) eggs			Unidentified (stage 1–3) eggs				Temp. (°C)
				Cod	Witch	Haddock	Total	Cod	Witch	Haddock	
H047	0		1981								
H011	0		1979								
H032	4	40	1980	0	0	0	0.11	0.11	0	0	−0.83
H050	11	84	1981	0	0	0	0.06	0.06	0	0	−0.25
H015	5	95	1979	0	0	0	0.50	0.50	0	0	−0.64
H035	12	129	1980	0	0	0	3.82	3.82	0	0	2.08
H018	5	137	1979	0.09	0	0	17.28	17.28	0	0	3.82
H036	11	156	1980	0.16	0	0	3.16	3.16	0	0	5.05
BR02	10	194	1980	0.08	0.09	0	0.58	0.29	0.30	0	10.97
VN01	7	219	1979	0.04	0.23	0	0.46	0.07	0.39	0	17.60
LE01	7	228	1980	0	0.05	0	0.04	0	0.04	0	14.10
VN02	7	239	1979	0	0	0	0.05	0	0.05	0	16.66
EK01	7	239	1981	0	0	0	0	0	0	0	15.49
LE02	7	249	1980	0	0	0	0.04	0	0.04	0	13.84
EK02	2	263	1981	0	0	0	0	0	0	0	14.60
H025	1	271	1979	0	0	0	0	0	0	0	13.00
AR03	12	279	1980	0.05	0.01	0	0.10	0.08	0.02	0	11.03
H028	7	320	1979	0	0	0	0.07	0.07	0	0	7.89
H045	3	349	1980	0	0	0	0.54	0.54	0	0	3.00

Material and Methods

The survey design and methods of sampling and analysing plankton, chlorophyll, and nutrients used during the SSIP were described by O'Boyle et al. (1984). Ichthyoplankton and zooplankton samples were all taken with 61-cm-diameter frame bongo nets using 0.333-mm Nitex mesh. Zooplankton counts were carried out on 755 of the 2061 SSIP stations included in the egg distribution analysis, and the information on seasonal cycles of zooplankton is therefore less complete.

Markle and Frost (1985) defined four egg stages; in the present analysis, their stages 1–3 were grouped together as "unidentified" and stage 4 as "identified" eggs. Some stage 3 eggs could in practice be identified, but for consistency, all stage 3 eggs were included in the "unidentified" category.

The proportion of eggs of the three species caught in a plankton sample depended on the area being sampled, the season, and the year. Variability due to these factors would be minimised by calculating the proportion of identified eggs of each species at each station and applying this to the unidentified eggs. However, because the number of identified eggs is small, there would be many stations with no identified eggs present and the estimated proportions would not be robust. The Scotian Shelf was therefore divided into 10 areas corresponding approximately to major banks and basins (Fig. 1) and the stations were grouped accordingly. The proportions of each species were calculated from the total number of identified eggs caught during each cruise in each area. The average number of stations within each area on each cruise was about 11, and sampling at all stations within an area was carried out within a few days. An example of the procedure for calculating species proportions is given for the Cape Breton area (Table 1).

The Cape Breton area is smaller and had fewer samples than other areas, with an average of only seven per cruise. There were over 30 times as many stage 1–3 eggs as stage 4 eggs in this area and no haddock were found. On several cruises, no stage 4 eggs occurred, and in these cases the identity of early-stage eggs was inferred from other cruises, which took place in the same area at similar times of year, or from stations in adjacent areas. For example, no identified eggs occurred in the Cape Breton area before day 137; only cod eggs were found between days 137 and 156 (Table 1). All eggs caught in this area during the earlier part of the year were therefore assumed to be cod. This kind of extrapolation was rare; over 90% of unidentified eggs were allocated to species using information from the cruise and area in which they were caught.

Because seasonal coverage in any one year was incomplete (Fig. 2), information from three years (1979–81), which had the most complete coverage and during which standardised sampling methods were used, was combined to give an average representation of the seasonal cycle throughout the year. Patchy sampling in time and space precluded analysis of variance as a means of calculating the proportion of variability in egg numbers due to area, season, and between-year effects.

Temperature differences between years (or other features of the environment, for which temperature is a proxy) might cause year to year variability in the magnitude or timing of egg production. However, temperature differences between 1979, 1980, and 1981 were small; all three were slightly warmer than the average for the period 1972–80 (Drinkwater and Trites 1988). Smith (1989) calculated temperature anomalies at three depths for two stations off southwestern Nova Scotia during this period. The differences in his anomalies for the three depths in 1979 were bigger than the differences between years, but on average, 1979 was slightly warmer than 1980 or 1981. Surface temperature data collected during SSIP cruises showed that during the period from day 120 to 240 the temperature in 1979 was slightly higher than in 1980. The rate of warming of surface water during the period from mid-April to late July was roughly 1° every 8 d (Fig. 3). Drinkwater and Trites (1987) found a similar rate for the Scotian Shelf during this time of year. Thus, a temperature difference of 1° between years corresponded to a timing difference of about 8 d, but there were "within-area" differences of several degrees during each cruise (Fig. 3). Given this variability and the variability in anomaly with depth, there was no reason to expect large differences in the timing or magnitude of egg production between the three years due to temperature.

The aggregated data for the three years, a total of 2061 samples collected during 19 cruises, were divided into short time periods in order to examine the seasonal timing of egg production in different areas. Again a balance was struck between the finest possible time resolution and the need to retain adequate numbers of samples in each time period. A time period of 20 d was chosen, which gave an average of 13 samples in each area in each time period. No data were available for any area during the first 20 d of the year or, more seriously, for the period from

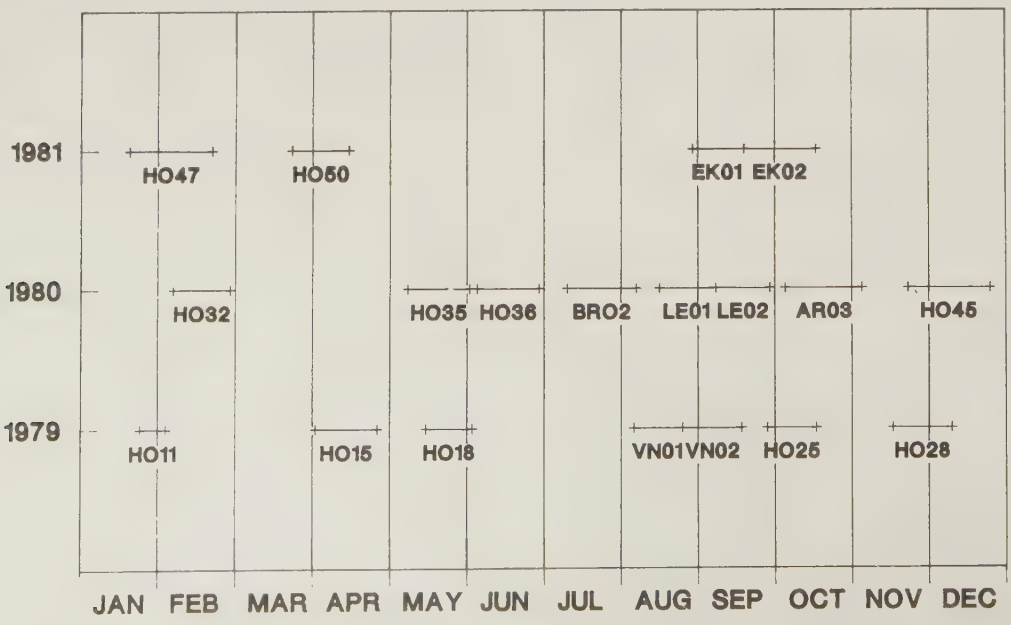

FIG. 2. Timing of SSIP cruises included in the analysis.

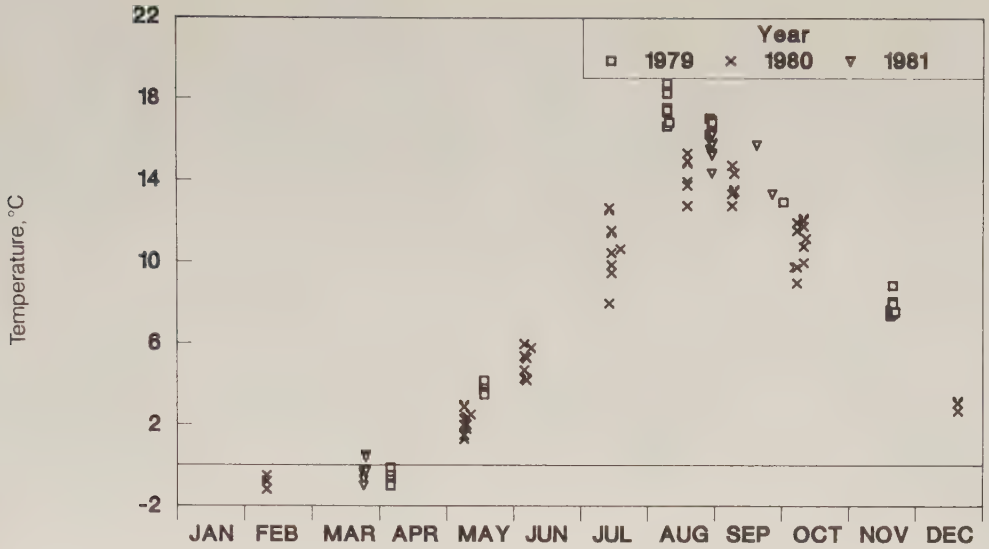

FIG. 3. Surface temperature at all stations sampled in the Cape Breton area during SSIP cruises in 1979–81.

day 60 to 79, which was the time when egg numbers were increasing rapidly in many areas.

Finally, the data for individual stations within each 20-d period were grouped into rectangles of 0.5° latitude by 1° longitude and averaged arithmetically to show the distributions of cod, haddock, and witch flounder over the whole area of the shelf.

Three measures of plankton production were examined for comparison with data on timing and location of cod spawning:

(1) Zooplankton counts (755 samples) provided information on the abundance of *Calanus finmarchicus*, but the mesh size used (0.333 mm) did not retain all of the smaller copepod species, nor early stages of *Calanus*. Frank (1988) pointed out the dangers of drawing conclusions about the co-occurrence of larvae and their prey because cod larvae feed mainly on early stages of *Calanus*. However, the data are used here to give an indication of the timing of *Calanus* production.

(2) Chlorophyll data were collected at most of the 2061 stations and provided an indication of the timing of primary production in the different areas. Timing of copepod production is dependent on the timing of primary production (Williams and Lindley 1980; Diel and Klein Breteler 1986; Colebrook 1979).

(3) Nitrate becomes depleted during the spring phytoplankton bloom, and nitrate level is therefore a measure of cumulative primary production in spring. For this reason, it may be a more useful indicator of the timing of primary production than chlorophyll data. Short-lived blooms are easily missed by infrequent sampling, such as the SSIP cruises.

Results

The principal aim of the paper is to reexamine the hypothesis that timing and location of cod spawning on the Scotian Shelf is coupled to the timing of plankton production, but the information on distribution of early-stage eggs of haddock and witch flounder is also of intrinsic interest and will be presented briefly.

Peak abundances of early-stage haddock eggs occurred in April and they were particularly abundant on Browns and Georges banks (Fig. 4). Spawning occurred as far north and east as Banquereau and Canso, but only sporadically, in May and June. No haddock eggs of any stage were taken after June and spawning began again in February, although it should be noted that no sampling was carried out south of La Have Bank in January during this 3-yr period.

Spawning of witch flounder took place between March and October, with large numbers of early-stage eggs being taken in June on Browns and Emerald banks and in the inshore area. As noted by Neilson et al. (1988), spawning was progressively later to the north and east, but unlike their distribution pictures of late-stage eggs, considerable numbers of early-stage witch flounder eggs were found close to the coast throughout the spawning period (Fig. 5).

Since the Cape Breton area was used above as an example of how species proportions were calculated, cod egg distributions in that area will be reviewed first. There were over 30 times as many stage 1–3 eggs as stage 4 eggs (Table 2). Early-stage eggs occurred at 36 stations and stage 4 eggs at only 11. Numbers of stage 1–3 eggs were greatest in samples taken around 11 May. By mid-July, very few were caught. The highest numbers of stage 4 eggs were found about 20 d after the peak in stages 1–3. Some stage 4 eggs were taken as late in the year as October, but these were isolated individual occurrences. The number of stage 1–3 eggs caught in July and August was an order of magnitude less than the number caught in May and June. In July the majority of these eggs were probably witch flounder, not cod. Cod spawning peaked in May in this area.

In spring, cod spawned over most of the Scotian Shelf, with greatest numbers of eggs on the offshore banks (Fig. 6). Sampling did not cover all times of year equally well (Fig. 2) and in some areas was not adequate to show seasonal timing. The best seasonal data coverage was obtained for the four areas Emerald, Sable, Banquereau, and Misaine (Table 2), and mean spawning dates for cod in these areas in the first half of the year were 24 March, 8 April, 9 April, and 3 May, respectively. These dates were calculated from egg abundance curves (Fig. 7), with an allowance for the average age of stage 1–3 eggs (7 d). The changes in abundance of *C. finmarchicus* in spring matched the cod egg curves closely and there is a similar shift in the timing of peak occurrence between the four areas

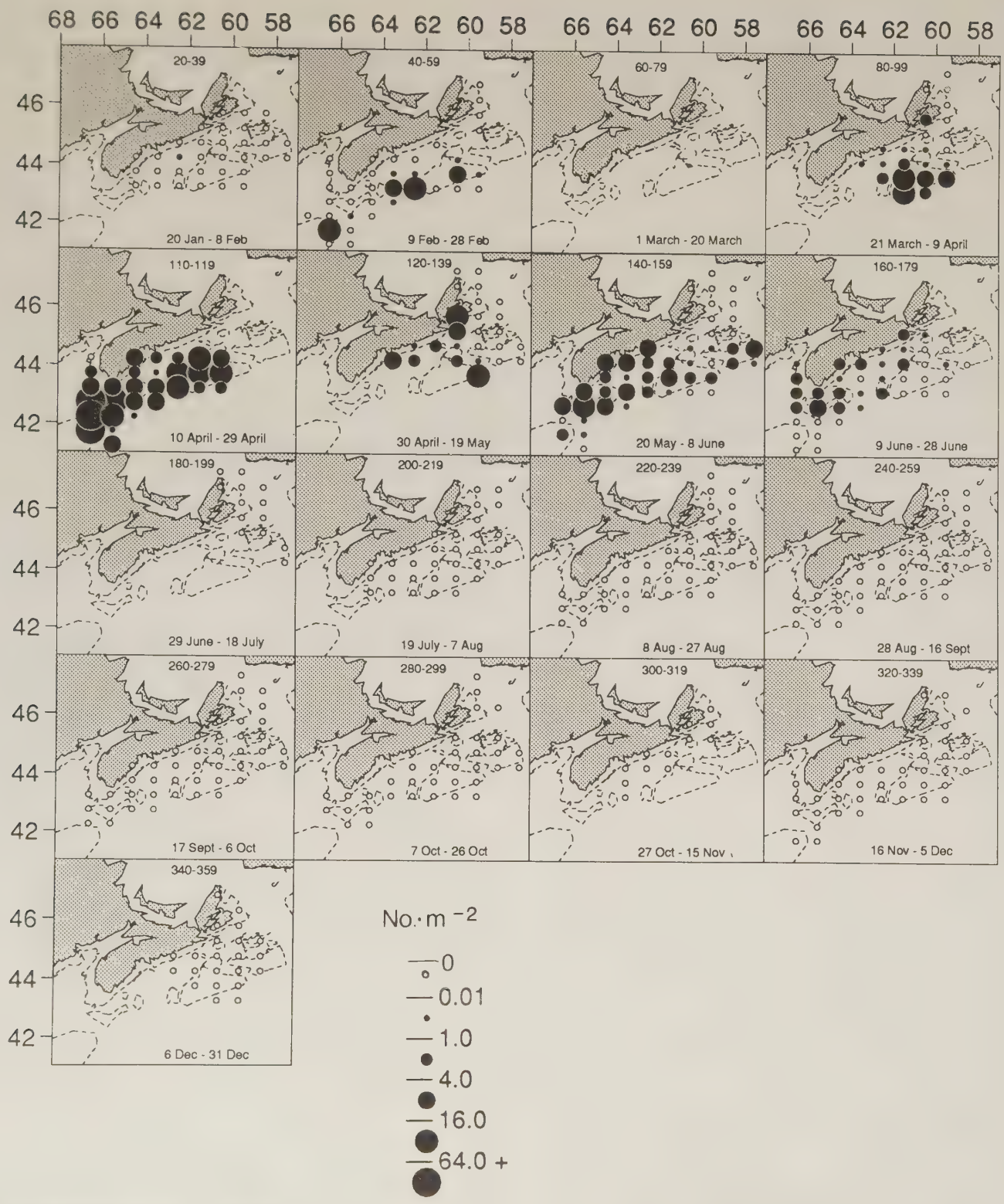

FIG. 4. Distribution of stage 1–3 haddock eggs. Data for 1979–81 have been combined and averaged arithmetically in 20-d periods for each 0.5° latitude by 1° longitude rectangle. The period from 1 to 20 March was not sampled. Sampling intervals are given as day of year at top and Gregorian date at bottom of each panel. Broken lines are the 100-m isobath.

(Fig. 7). A linear regression of cod egg abundance on *Calanus* abundance for the 518 stations at which both were counted during the first 6 mo of the year gave a significant positive correlation coefficient ($r = 0.14$, 516 df, $p < 0.01$). This is not a rigorous test of the hypothesis that the timing of cod spawning in spring is linked to the timing of *Calanus* production, for reasons which will be given in the discussion, but it suggests that the possibility of such linkage cannot be excluded and should be explored further.

In fall the distribution of cod eggs was more restricted than in spring. The main spawning area extended from the Gully east of Sable Island, across Middle Bank, and into the inshore

FIG. 5. Distribution of stage 1–3 witch flounder eggs. Data handled as for haddock.

area near to and west of Halifax (Fig. 6). Very little spawning took place in Emerald or Misaine. *Calanus* abundance did not increase in the fall, although it remained fairly high throughout the second half of the year in Misaine and Banquereau (Fig. 7). *Calanus* were more abundant on the northern Scotian Shelf (north of 44°N) during the period October–December (Fig. 8).

A linear regression of cod egg abundance on *Calanus* abundance for the 197 stations at which both were counted during the second 6 mo of the year gave a nonsignificant negative correlation coefficient ($r = -0.08$, 195 df, $p > 0.05$). In the fall, cod did not spawn in all areas where *Calanus* were abundant (e.g. Misaine, Fig. 4) and other factors may be involved. Information on chlorophyll and nitrate levels will be examined next to give an idea of the seasonal cycle in primary production and in particular for evidence of an autumn bloom.

In all four areas, highest spring chlorophyll levels were recorded before the end of April, but the timing of the bloom could not be determined precisely. Nitrate levels in Emerald

TABLE 2. Numbers of cod, witch flounder, and haddock eggs per square metre and mean surface temperature in 20-d periods for the Cape Breton, Emerald Bank, Sable Bank, Banquereau, and Misaine Bank areas.

20-d period	Number of stations	Day of year	Identified (stage 4) eggs			Unidentified (stage 1–3) eggs				Temp. (°C)
			Cod	Witch	Haddock	Total	Cod	Witch	Haddock	
					Cape Breton					
30	0									
50	4	40	0	0	0	0.11	0.11	0	0	−0.83
70	0									
90	16	87	0	0	0	0.20	0.20	0	0	−0.38
110	0									
130	17	131	0.03	0	0	7.78	7.78	0	0	2.59
150	11	156	0.16	0	0	3.16	3.16	0	0	5.05
170	0									
190	10	194	0.08	0.09	0	0.58	0.29	0.30	0	10.97
210	6	219	0.04	0.27	0	0.53	0.08	0.46	0	17.72
230	22	235	0	0.02	0	0.03	0	0.03	0	15.48
250	8	250	0	0	0	0.04	0	0.04	0	14.09
270	7	274	0.03	0	0	0	0	0	0	11.23
290	7	280	0.05	0.02	0	0.17	0.14	0.03	0	11.46
310	2	319	0	0	0	0	0	0	0	7.55
330	5	320	0	0	0	0.09	0.09	0	0	8.02
350	3	349	0	0	0	0.54	0.54	0	0	3.00
					Emerald Bank					
30	18	31	0.02	0.00	0	1.50	1.50	0	0	3.49
50	7	51	0	0	0	19.74	4.93	0	14.80	3.86
70	0									
90	0									
110	18	104	0.99	0	4.46	75.07	16.91	0	58.16	3.63
130	0									
150	15	146	0	0.51	0.19	8.86	0	6.16	2.70	8.71
170	9	171	0	1.13	0.24	8.33	0	6.88	1.45	10.38
190	0									
210	9	214	0	0.11	0	0.86	0	0.86	0	16.48
230	14	231	0	0	0	0.53	0	0.53	0	16.96
250	21	247	0	0	0	0.06	0	0.06	0	17.13
270	19	272	0	0	0	0.24	0	0.24	0	15.44
290	16	291	0	0	0	0.30	0	0.30	0	14.19
310	0									
330	10	333	0	0	0	0.91	0.91	0	0	10.64
350	0									
					Sable Bank					
30	27	29	0.08	0	0	1.36	1.36	0	0	2.77
50	12	44	0	0	0	4.95	3.12	0	1.83	1.72
70	0									
90	21	95	0.91	0	0.18	25.41	14.88	0.01	10.53	3.48
110	19	104	1.88	0.04	3.06	30.09	12.83	0.24	17.02	3.66
130	3	135	1.10	0	0	34.04	24.49	0	9.56	4.87
150	32	145	0.33	0	0.31	3.40	1.27	0	2.12	8.07
170	20	166	0	0.11	0	1.02	0	1.02	0	9.27
190	0									
210	20	211	0	0.32	0	0.88	0	0.88	0	16.43
230	40	231	0	0.35	0	0.67	0	0.67	0	16.72
250	57	249	0	0.09	0	0.21	0	0.21	0	17.74
270	32	275	0	0	0	1.64	1.64	0	0	15.00
290	32	291	0.15	0	0	1.89	1.89	0	0	13.54
310	0									
330	20	330	0.69	0	0	6.47	6.47	0	0	11.12
350	14	343	4.22	0	0	20.76	20.76	0	0	5.72
					Banquereau					
30	17	27	0	0	0	0.75	0.75	0	0	1.68
50	0									
70	0									

TABLE 2. (*Concluded*)

20-d period	Number of stations	Day of year	Identified (stage 4) eggs			Unidentified (stage 1–3) eggs				Temp. (°C)
			Cod	Witch	Haddock	Total	Cod	Witch	Haddock	
90	13	94	0.06	0	0	10.05	10.05	0	0	1.85
110	0									
130	17	132	0.81	0	0	16.17	16.17	0	0	3.65
150	23	152	0.08	0	0.05	3.80	2.23	0	1.57	6.15
170	7	160	0	0	0	0.04	0.01	0	0.03	6.47
190	3	196	0	0	0	0.57	0	0.57	0	11.30
210	8	217	0	0.44	0	1.42	0	1.42	0	16.91
230	39	230	0	0.17	0	0.44	0	0.44	0	15.69
250	40	247	0	0.02	0	0.18	0	0.18	0	16.57
270	26	269	0	0.06	0	0.26	0.02	0.25	0	14.87
290	18	284	0.03	0	0	0.49	0.49	0	0	12.83
310	0									
330	13	325	0.67	0	0	7.35	7.35	0	0	8.95
350	12	347	0.29	0	0	9.97	9.97	0	0	4.04
					Misaine Bank					
30	16	24	0	0	0	0.03	0.03	0	0	0.36
50	10	41	0	0	0	0.03	0.03	0	0	−0.13
70	0									
90	25	91	0	0	0	0.82	0.82	0	0	0.50
110	0									
130	18	133	0.23	0	0	6.63	6.63	0	0	3.06
150	16	156	0.03	0	0	3.46	3.46	0	0	5.63
170	1	160	0	0	0	0	0	0	0	6.80
190	13	198	0	0.12	0	4.77	0	4.77	0	11.48
210	10	218	0.04	0.41	0	2.54	0.21	2.33	0	17.22
230	29	233	0.01	0.02	0	0.38	0.09	0.28	0	16.03
250	19	248	0	0.04	0	0.38	0	0.38	0	15.90
270	29	270	0	0	0	0.01	0	0.01	0	14.28
290	9	283	0	0	0	0	0	0	0	11.23
310	3	319	0	0	0	0	0	0	0	9.57
330	9	324	0	0	0	0	0	0	0	8.11
350	10	347	0	0	0	0.14	0.14	0	0	3.42

and Sable dropped to less than 1 $\mu mol \cdot L^{-1}$ by 20 April whereas in Banquereau and Misaine, nitrate remained above this level until the end of May (Fig. 9). The main period of phytoplankton production was probably earlier in the former two areas. Fournier et al. (1977) found a high level of primary production and low nitrate on Emerald Bank in early March.

Chlorophyll values increased in all areas in October, with little difference in timing between areas (Fig. 9). Surface nitrate values did not increase until December, well after the autumn bloom, presumably because of the dynamic balance between upwelling nitrate and uptake by phytoplankton.

The principal hypothesis examined here is that cod spawning is linked to copepod production, but an alternative hypothesis might be to ascribe differences in timing between areas to temperature. Cod spawned over a fairly wide temperature range (Fig. 10) and the data did not support the hypothesis that spawning occurred when a particular water temperature was attained. Spring spawning began at a time when there were consistent surface temperature differences of up to 4° between the four areas (Table 2). Surface temperature is representative of the upper 50 m of the water column until May, when thermal stratification develops (Drinkwater and Trites 1987; McLaren et al. 1989). Cod spawning began and, on Emerald Bank, peaked during a period when temperature was not increasing; therefore, it is unlikely that spawning was triggered by a change in temperature. Temperature changed very little until April and after that the rate of warming was approximately the same in all areas. In fall, early-stage cod eggs were found at surface water temperatures about 5° higher than in spring, but since surface temperature in fall may not be representative of the depth range over which the eggs occur, it would be unsafe to draw any conclusions. The issue will be raised again briefly in the discussion. One cannot exclude the possibility that differences in timing of spawning between areas arise because of the effect of temperature on the maturation of spawning fish.

Discussion

The distributions of early-stage cod, haddock, and witch flounder eggs presented here depend on the assumption that the species proportions of late-stage (identified) eggs are representative of the species composition of early egg stages within the same cruise and area. Three factors which may affect this relationship are (a) differences in temperature-dependent development rate between species, (b) differences in egg mortality, and (c) the time lag between early- and late-stage eggs. Development rates of cod and haddock eggs are known to be very close (Page and Frank 1989) and witch flounder also have similar rates (Bigelow and Schroeder 1953). There is no comparative information on egg mortality rates for the three species. In principle it would be possible to calculate egg production curves for each species in each area from the late-stage eggs in order to allow for the time lag between early- and late-stage eggs, but the number of late-stage eggs is too small and the

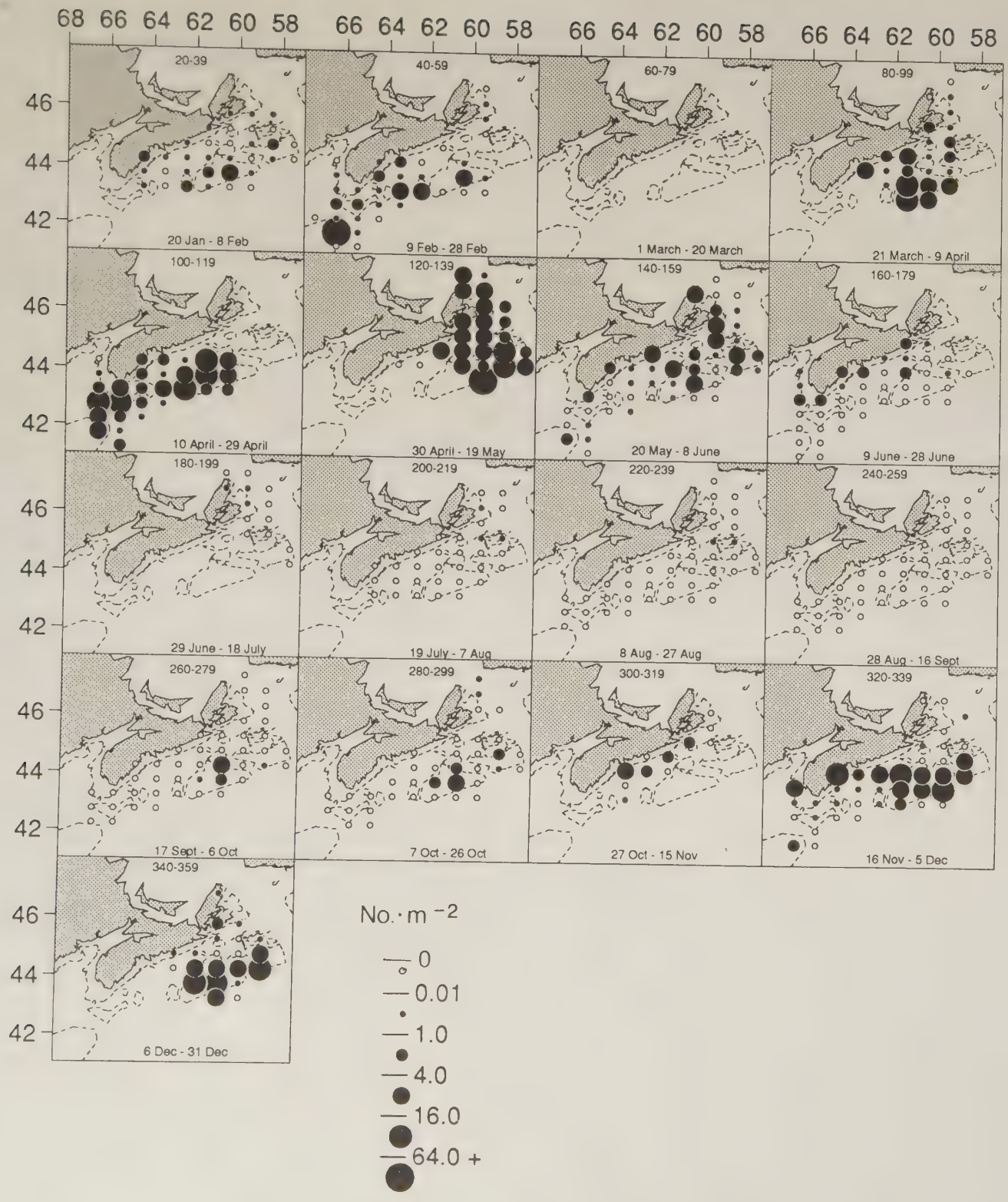

FIG. 6. Distribution of stage 1–3 cod eggs. Data handled as for haddock.

time resolution of the SSIP sampling is inadequate for such refinements.

Another possible cause of error is that eggs may be transported from one area to another during their development. The magnitude of this error can be related to the size of the area, the rate of advection, and the time taken to develop. Neilson et al. (1988) calculated the maximum distance that witch flounder eggs are likely to be transported prior to hatching as just under 14 km, using an average residual current of 2 $cm \cdot s^{-1}$ (based on Drinkwater et al. 1979). Applying this residual to cod and haddock eggs, the maximum distance travelled between spawning and hatching is about 35 km; between spawning and the average age of the unidentified stage (7 d), it is 12 km, and between the unidentified stage and the beginning of stage 4, it is 19 km. This gives a rough indication of the magnitude of displacement of the unidentified and identified egg distributions

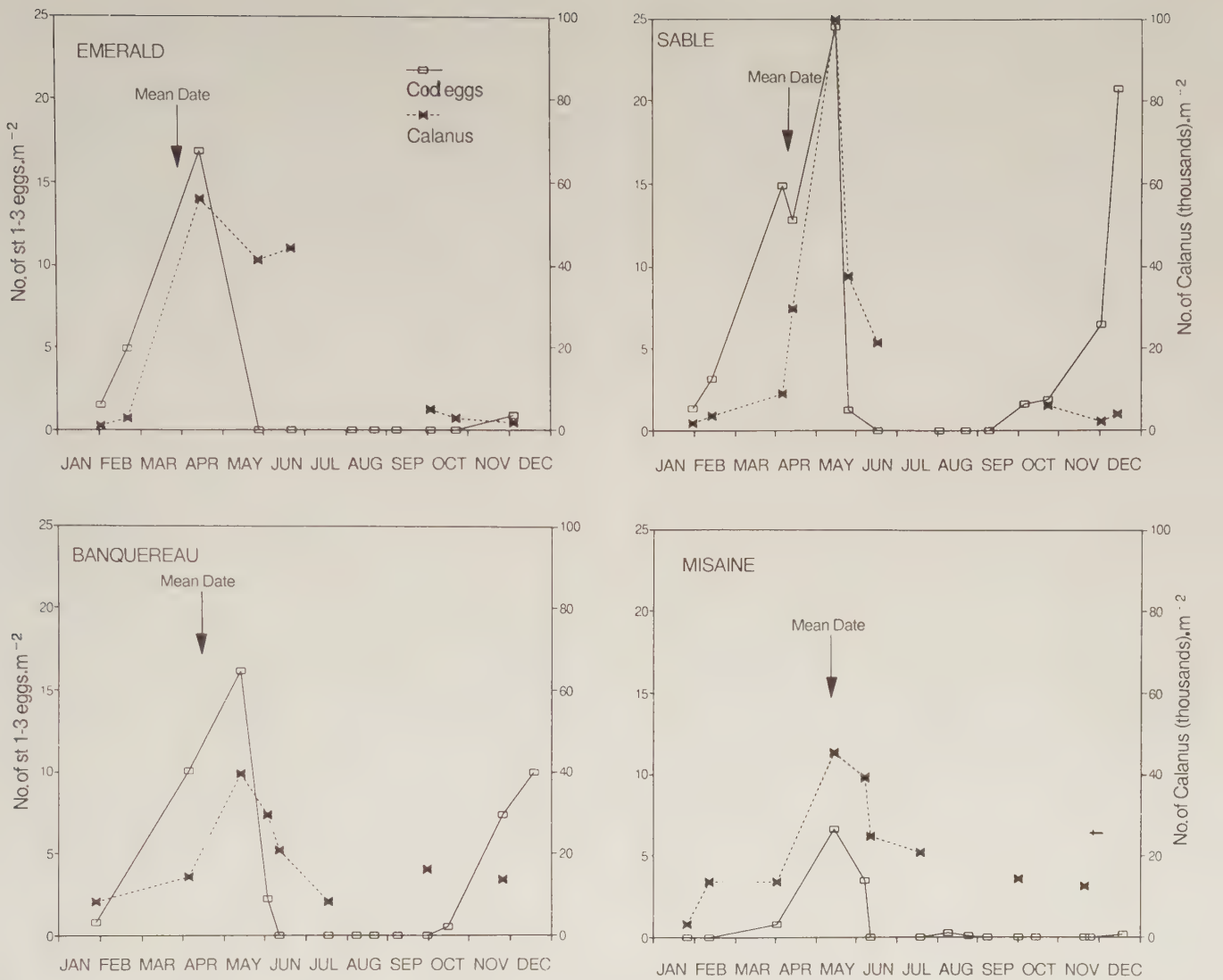

FIG. 7. Numbers of stage 1–3 cod eggs and *C. finmarchicus* per square metre, averaged arithmetically in 20-d periods for Emerald, Sable, Banquereau, and Misaine. Data for the years 1979, 1980, and 1981 have been combined. The averages of the dates of sampling within each 20-d period have been used in the plots, not the midpoint. The arrowed date is the mean date of cod spawning.

from the original spawning location, but it may be an underestimate of displacement in many cases, since rates of advection are variable and considerably higher in some parts of the Scotian Shelf, such as the inshore area (Gregory and Smith 1988). The dimensions of the rectangles in which the data are grouped in Fig. 4, 5, and 6 are about 60 by 42 km, but the areas used to calculate the species proportions are much larger and therefore less affected by transport.

Although a direct method for identifying individual eggs would be preferable, useful additional information on location and timing of spawning of cod, haddock, and witch flounder can be derived from the two-stage procedure used here. Authors who draw conclusions about spawning distribution from late-stage eggs (e.g. O'Boyle et al. 1984; Gagné and O'Boyle 1984; Neilson et al. 1988) are making several of the same assumptions, but they are using only the oldest 3–4% of the total eggs sampled, and older eggs will have been transported further from their spawning origin. An alternative estimate of species distribution might be obtained by means of fishing surveys or commercial catches, but detailed sampling of maturity states would be needed to estimate spawning dates.

This is not the first time that data from SSIP have been used to describe location and timing of spawning of cod, haddock, and witch flounder on the Scotian Shelf, but it is the first attempt to interpret the distribution of early-stage eggs of these three species. A very important conclusion from previous descriptions (O'Boyle et al. 1984; Gagné and O'Boyle 1984) was that cod on the Scotian Shelf spawned over long periods of the year and the timing did not match with the time of seasonal blooms, in contrast with the situation found in other cod stocks (e.g. Arcto-Norwegian, Ellertsen et al. 1987).

The first published analysis of the temporal and spatial distribution of ichthyoplankton communities of the Scotian Shelf using SSIP data (O'Boyle et al. 1984) looked at six species from 11 cruises. Only late-stage eggs and larvae of cod and haddock were included because of the identification problem. O'Boyle et al. (1984) stated that cod spawning began in late autumn (November) on Sable Bank and continued through to April on Emerald, Western, and Browns banks. They concluded that "eggs and larvae of cod (and pollock) are abundant during midwinter and spring, prior to the initiation of the plankton growth season." This conclusion seems to be erroneous.

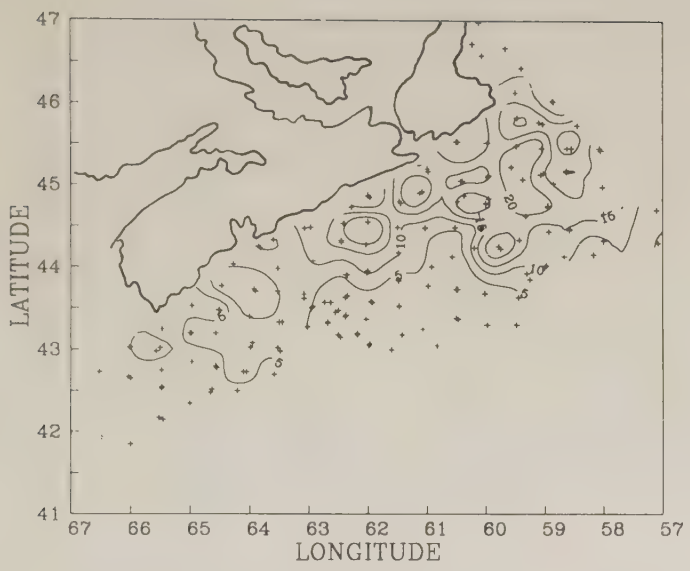

FIG. 8. Number (thousands per square metre) of *C. finmarchicus* during October–December.

Table 3 of Gagné and O'Boyle (1984) shows that late-stage cod eggs are absent in all areas in January and February and larvae are only present in a few, at low levels. Also, our Fig. 7 shows that early-stage eggs on Sable Bank (and elsewhere) occur in two quite discrete peaks, one in December and one in May.

The second analysis of the timing of cod spawning on the Scotian Shelf by Gagné and O'Boyle (1984) used data on late-stage eggs and larvae from 16 SSIP cruises during the period 1979–81 and also discussed distributions of 1-yr-old cod from Canadian groundfish surveys. Their description of the pattern of spawning is substantially similar to our Fig. 6. Major spawning activity occurred over most of the Scotian Shelf during the period March–May, and in some areas there was a short, but intense period of spawning in November–December. Gagné and O'Boyle (1984) concluded that, although cod spawning and plankton production timing are closely coupled in spring, the summer and fall spawning on the Scotian Shelf do not fit this hypothesis:

(1) "It is much more difficult to suggest a relationship between the bloom and the spawning activities in the fall since the major concentrations of larvae are found in November and December, well after the bloom. This suggests that seasonal blooms are not essential for the achievement of intense spawning by cod."

(2) "This is further exemplified by the situation along the Laurentian Channel where cod seem to spawn extensively in summer, right between the two seasonal blooms."

These are the two main pieces of evidence against the match hypothesis and thus need to be evaluated carefully. The second point has already been dealt with to some extent, when discussing the results for the Cape Breton area. Although it is true that early-stage cod eggs are sometimes present during the period June–August along the Laurentian Channel (and not elsewhere, see Fig. 6), the numbers are insignificant when compared with the numbers of early-stage eggs found in May (Table 2, and Cape Breton and Misaine). The evidence from the Cape Breton area and from Misaine, which also borders the Laurentian Channel, supports the hypothesis that the timing of cod spawning and plankton production are coupled rather than contradicting it (Fig. 7).

We now turn to the first point, whether fall spawning of cod is coupled with a seasonal increase in plankton. Our reanalysis of the SSIP data provides better information on the location and timing of fall spawning than was available to Gagné and O'Boyle (1984), and there is some additional information on copepod production from other sources. Cod egg abundance and *Calanus* abundance were not correlated in the second half of the year, but, as discussed below, this result should be treated cautiously. Cod spawning was confined to parts of the northern half of the Shelf in November and December (Fig. 6). *Calanus finmarchicus* were more abundant over the northern part of the Scotian Shelf at this time of year (Fig. 8). An unpublished account of the seasonal timing of copepod production in the Northwest Atlantic from 10 yr of Continuous Plankton Recorder (CPR) data by Allan Longhurst (Bedford Institute, pers. comm.) indicates a single generation of early copepodites of *C. finmarchicus* in the eastern Gulf of Maine and southern Scotian Shelf, but two generations (early summer and fall) on the northern Scotian Shelf. Further south, on Emerald Bank, McLaren et al. (1989) showed increased copepod production between September and December, dominated by *Centropages typicus* and *Metridia lucens*. The autumn phytoplankton bloom would be expected to give rise to increased copepod production.

There is considerable evidence that fall spawning of cod could be coupled to increased copepod production. In spite of the lack of correlation between cod eggs and *Calanus*, it would be premature to reject the match hypothesis because the evidence to date is inconclusive. Several issues must be addressed in order to allow a rigorous test to be made:

(1) Measurements of production rates rather than abundances of copepod species are required. *Calanus finmarchicus* is the major food species of cod larvae over most of its range (e.g. Ellertsen et al. 1987; Sherman et al. 1984), but other copepod species may be important at the southern end (Thompson and Harrop 1991).

(2) Adequate estimates of the timing of production and spawning require more frequent sampling than was the case with SSIP. In fact, it is questionable whether a purely empirical estimate of production timing is appropriate and an alternative would be to model the processes governing timing of copepod production. The factors would include primary production, temperature, and overwintering population.

(3) It is unlikely that copepod production is a sufficient condition for cod spawning even if it is a necessary one. No cod spawning occurred in Misaine in the fall, although this was an area where *Calanus* abundance remained high. Other factors may also be involved and the lack of correlation between cod egg numbers and *Calanus* abundance in the fall, reported above, does not lead to rejection of the hypothesis.

(4) Unlike haddock, the date of cod spawning within an area is apparently not affected by year to year variability in physical factors (Page and Frank 1989; Pedersen 1984). This suggests that in cod, but perhaps not in haddock, the timing of spawning is adapted to the average timing of plankton production rather than the timing in a particular year. A test of the match hypothesis for cod should therefore compare average spawning time with average production timing.

MacCall (1990) has suggested a number of ways in which his "basin model" could be used to explore optimal timing for a single-batch spawner in relation to seasonal changes in "habitat suitability." In the context of the match hypothesis, "habitat suitability" for cod larvae would be measured by the production of certain size categories or life stages of particular

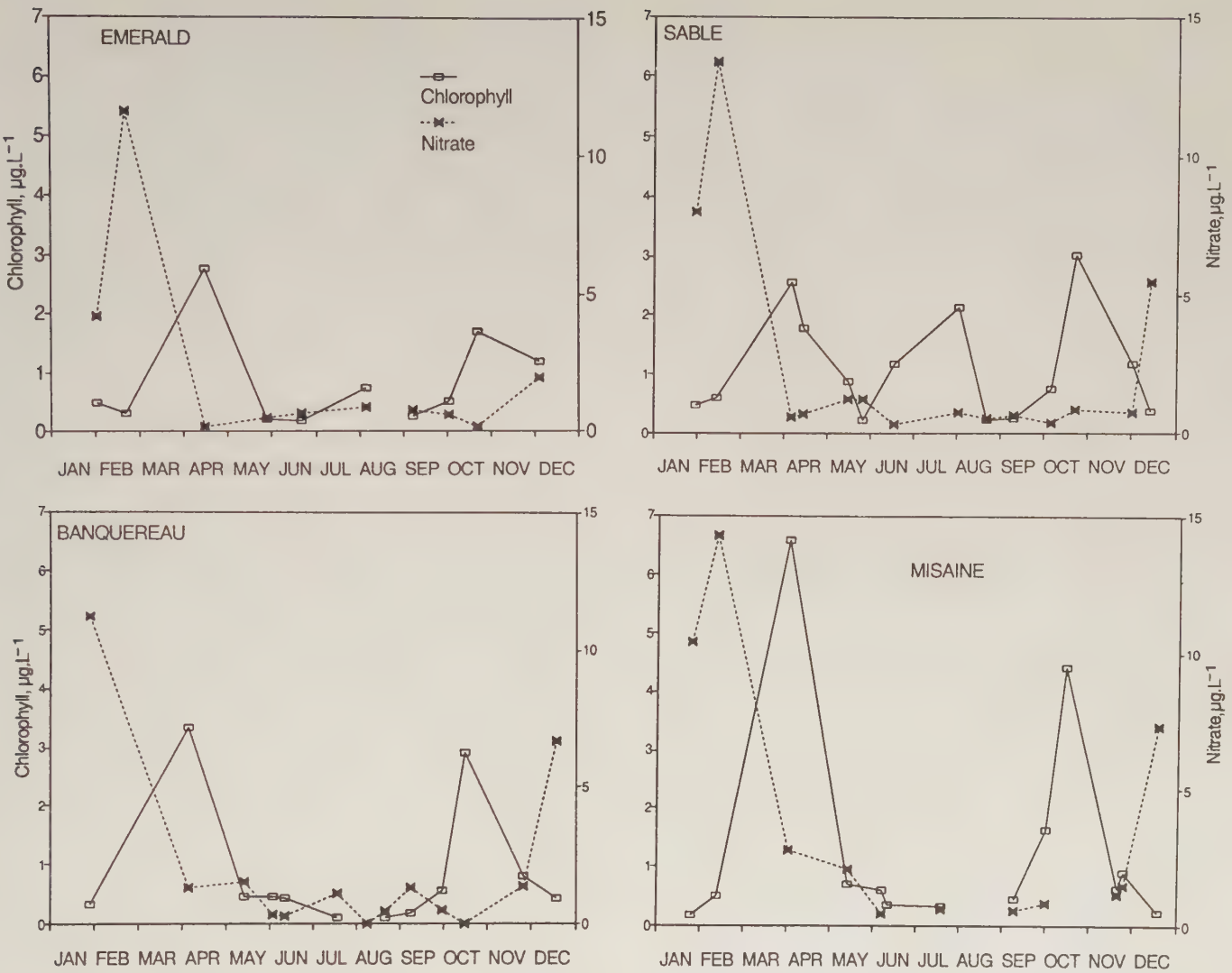

FIG. 9. Surface chlorophyll and surface nitrate averaged arithmetically in 20-d periods for Emerald, Sable, Banquereau, and Misaine.

copepod species, which are known to be their prey (Frank 1988). Time lags between cod spawning and plankton production could then be calculated precisely (e.g. Ellertsen et al. 1987). MacCall (1990) pointed out that "The problem of optimal timing of single-batch spawning becomes much more interesting as complications are added: What is the optimal strategy if the optimum time cannot be anticipated accurately? How is the optimum strategy altered if larger, early spawned larvae have a competitive advantage over (or even cannibalize) younger larvae?"

As discussed earlier, an alternative hypothesis to account for differences in timing of spawning of cod between areas is that they are due to temperature, but there are reasons for doubting this or at least for suspecting that the relationship is indirect. If the alternative hypothesis were true, then one would expect that the timing of spawning within an area would vary from year to year due to differences in temperature. Long-term evidence on the timing of spawning in Arcto-Norwegian cod (e.g. Pedersen 1984) shows that it varies very little in date, in spite of year to year temperature variability. This may be partly because year to year differences in temperature are small compared with differences through the water column. In contrast, the timing of haddock spawning varies between years on Browns Bank (Page and Frank 1989; Hurley and Campana 1989) and haddock spawning timing is affected by temperature on Georges Bank (Marak and Livingstone 1970; Waiwood and Buzeta 1989).

Cod stocks in the North Atlantic spawn over a wide range in temperature between about −1 and 9°C and there is an inverse relationship between temperature and vertebral count (Brander 1979). Vertebral counts within a stock vary from year to year, depending on the water temperature (Brander 1979; Lear and Wells 1984), which indicates that spawning does not take place at a fixed temperature. Vertebral counts of cod from different areas on the Scotian Shelf show consistent differences, with a cline of increasing vertebral count on the offshore banks, going from southeast to northwest. Autumn-spawning cod off Halifax have a characteristically low vertebral count, which is presumably indicative of the higher temperature encountered during their early development (Mackenzie and Smith 1955).

A third hypothesis, which has been invoked to explain the spatial distribution of cod eggs and larvae in relation to banks on the Scotian Shelf, is the "retention" hypothesis (O'Boyle et al. 1984; Gagné and O'Boyle 1984). This explanation has been investigated thoroughly in southwestern Nova Scotia (Suthers and Frank 1989) and is not considered here, except to point out that "retention" is used to explain spawning location,

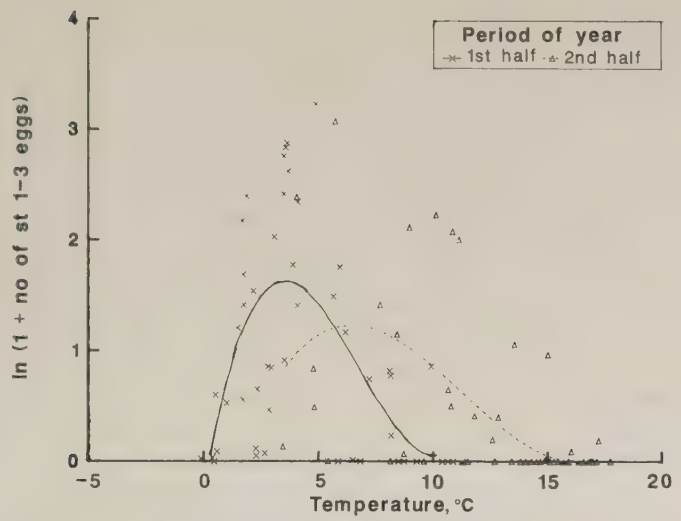

FIG. 10. Relationship between temperature and stage 1–3 cod eggs for the whole Scotian Shelf. The year has been divided into two halves at 28 June. Numbers are arithmetic averages for each of the 10 areas in each 20-d period, which are then log transformed. Curves are a cubic function, fitted by least squares.

but not timing. The present paper is mainly concerned with timing of cod spawning and with differences in timing between different areas.

We conclude that it is premature to reject the match hypothesis for cod spawning on the Scotian Shelf; the available evidence in many respects supports the hypothesis, but is not definitive. Given the methodological and empirical difficulties touched on above, one may question whether the match hypothesis can in practice be tested and whether it is important to do so. On both, we are inclined to give a positive answer. The hypothesis would be refuted if, for example, it were shown that timing of copepod production over the Scotian Shelf did not match observed differences in timing of cod spawning (e.g. a 40-d difference between Emerald Bank and Misaine in spring) or if it could be shown that copepod production does not increase in fall in the areas and at the times where cod spawn. It is important to test the hypothesis in order to establish whether the availability of food for cod larvae is a key factor shaping their life history. Also, the second part of the match–mismatch hypothesis (variability in timing of plankton production leads to variability in larval mortality and hence in year class strength) cannot hold if the first part (the match hypothesis) is refuted. The diversity of the Scotian Shelf provides a challenge when looking for unifying explanations for the observed location and timing of spawning. Copepod production may be one of the necessary components for survival of cod larvae and hence may exert selective pressure on the choice of spawning sites and timing by adult cod.

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Interpretation of Radiophosphate Dynamics in Lake Waters

T. R. Fisher

Horn Point Environmental Laboratories, University of Maryland-CEES, Cambridge, MD 21613, USA

and D. R. S. Lean

Environment Canada, National Water Research Institute, Box 5050, Burlington, Ont., L7R 4A6, Canada

Fisher, T. R., and D. R. S. Lean. 1992. Interpretation of radiophosphate dynamics in lake waters. Can. J. Fish. Aquat. Sci. 49: 252–258.

Models of planktonic phosphorus dynamics over the last 30–40 yr depend on the steady-state distribution of isotope for the determination of compartment size. Radiophosphate data for P-deficient lakes in summer have shown a steady-state distribution of 1–15% of ^{32}P in the filtrate within 0.5–5 h. To explain this, a phosphate back-flux term from the particulate fraction has been widely accepted (phosphate is believed to be released from the internal pools of phosphate consumers and by excretion from herbivores and bacterivores). We show that dialysis of lake water at isotopic steady state provides values for the dissolved $[^{32}P]PO_4$ compartment up to an order of magnitude lower than those obtained by membrane filtration and gel filtration chromatography. This apparently occurs as a result of minor cell damage during filtration when most of the $[^{32}P]PO_4$ is in the particulate pool. Consequently, the size of the phosphate pool and the magnitudes of phosphate uptake and back-flux may have been overestimated by up to a factor of 10. Furthermore, the turnover time of the particulate compartment lengthens from ~40 min to >1 d, which is more consistent with models describing P fluxes between functional components of the plankton.

Les modèles de la dynamique du phosphore planctonique élaborés au cours des 30 à 40 dernières années dépendent de la distribution à l'état d'équilibre d'isotopes pour la détermination de la taille des compartiments. Des données sur le radiophosphate dans des lacs carencés en P en été ont révélé une distribution à l'état d'équilibre allant de 1 à 15 % de ^{32}P dans le filtrat en deçà de 0,5 à 5 h. Afin d'expliquer ce phénomène, on reconnaît généralement qu'il y a un reflux du phosphate de la fraction particulaire. On croit ainsi que le phosphate est libéré des réserves internes des consommateurs de phosphate de par l'excrétion des herbivores et des bactérivores. Les auteurs montrent que la dialyse d'eau de lac en état d'équilibre isotopique fournit des valeurs pour le compartiment $[^{32}P]PO_4$ dissous jusqu'à un ordre de grandeur inférieures à celles obtenues par filtration sur membrane et par chromatographie par perméation sur gel. Ceci semble s'expliquer par de légers dommages aux cellules lors de la filtration, lorsque la plus grande partie du $[^{32}P]PO_4$ est retenue dans les particules. Par conséquent, la taille de la réserve de phosphate et l'ampleur de l'absorption et du reflux du phosphate peuvent avoir été surestimées par un facteur allant jusqu'à 10. De plus, le temps de transit du compartiment particulaire passe d'environ 40 min à plus d'une journée, ce qui concorde mieux aux modèles décrivant les flux de P entre les composantes fonctionnelles du plancton.

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A paradigm changed when $[^{32}P]PO_4$ was added to lake water samples. It was discovered that orthophosphate (hereafter, PO_4) was taken up in a matter of minutes under P-limited conditions (Hayes et al. 1952; Rigler 1956). Since this original observation, rapid turnover times have been observed in many lakes throughout the world (e.g. Rigler 1973; Lean 1973a, 1973b; Peters and MacIntyre 1976; Levine and Schindler 1980; Peters 1983; Prepas 1983; Pick et al. 1984; Lean et al. 1983, 1987; Istvanovics and Herodek 1985; White et al. 1985; Tarapchak and Herche 1986; Taylor and Lean 1991; and references therein).

Rapid PO_4 turnover times suggest a high demand for a small ambient pool. However, the measurements with labelled PO_4 created an apparent contradiction. Isotopic steady state was often achieved between particulate and dissolved compartments within 0.5–5 h, yet 1–15% of the ^{32}P activity remained in the filtrate. Therefore, a back-flux of P was required to balance uptake in an apparent steady state. But why would plankton release the growth-limiting nutrient? Originally, the observed redistribution of isotope at steady state was thought to be consistent with the two-compartment model illustrated schematically in Fig. 1 (Rigler 1973) and described mathematically as

$$(1) \quad k_1 \cdot a_1 = k_2 \cdot a_2$$

where k_1 is the uptake rate constant (per minute), a_1 is the dissolved activity (disintegrations per minute (dpm) per millilitre) at steady state, k_2 is a back-flux rate constant (per minute), and a_2 is the particulate activity (dpm per millilitre) at steady state. To simplify, the particulate phosphorus (PP) compartment was considered to be homogeneous and exchanging with a single dissolved PO_4 compartment. The uptake and back-flux terms were obtained from the radioisotope data and, when divided by the specific activity of their respective pool concentrations (dpm per nanomole), yielded mass fluxes per unit volume and time (nanomoles per millilitre per minute).

However, it soon became clear that the molybdate test overestimated the biologically available PO_4 by factors of 10–100 (e.g. Rigler 1966). As a result, concentrations were then

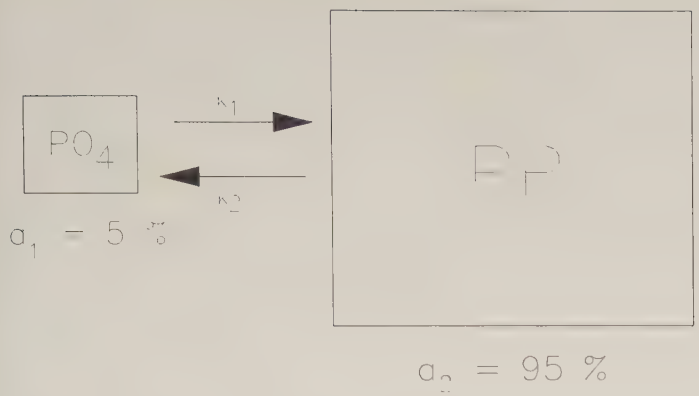

FIG. 1. Original isotope model for $[^{32}P]PO_4$ dynamics in lacustrine plankton (Rigler 1956). Terms a_1 and a_2 = ^{32}P activity in the orthophosphate (PO_4) and particulate P (PP) pools at isotopic steady state; k_1 and k_2 = fractional rate constants (per minute) for transfer of isotope between pools.

expressed as a fraction of the total activity, and k_1, the uptake rate constant, represented uptake of radiophosphate by phytoplankton and bacteria. The back-flux rate constant, k_2, included release from the primary PO_4 consumers (phytoplankton and bacteria) and regeneration by excretory activities of herbivores and bacterivores in the pool.

The turnover time (T) of each pool was also a useful concept. Turnover times (minutes) of the particulate and dissolved compartments are the reciprocals of the rate constants (per minute). Rearranging eq. (1) and substituting T_1 or T_2 for $(k_1)^{-1}$ or $(k_2)^{-1}$ yields

$$(2) \quad T_2 = T_1 ([f_1]^{-1} - 1)$$

where f_1 is the fraction of the total activity in the dissolved compartment at isotopic steady state, or $a_1/(a_1 + a_2)$. Using a typical value of 2 min for the turnover time of the dissolved pool (T_1) under summer P limitation and a 5% residual activity in the dissolved pool at steady state ($f_1 = 0.05$), the estimated turnover time for the PP compartment (T_2) is a brief 38 min. Could all of the PP pool really be replaced, on average, every 38 min under summer P-limited conditions?

The two-compartment model was modified by the results of Lean (1973a, 1973b). Using gel permeation chromatography, it was shown that only 1/10 of the ^{32}P-labelled material in filtrate at steady state was PO_4. An unidentified compound (XP) of small molecular weight (~250) appeared shortly after addition of $[^{32}P]PO_4$ to whole lake water but not filtered lake water. XP then combined with colloidal material, producing colloidal ^{32}P (CP) and releasing $^{32}PO_4$ (Fig. 2). CP had a high apparent molecular weight (>5 000 000) and may represent, in part, bacteria or other particulates which could pass a 0.45-μm filter. Furthermore, over 80% of the filtrate ^{32}P was CP.

The relative magnitudes of several of the fluxes in Fig. 2 were evaluated by Lean (1973a, 1973b). Uptake of PO_4 into the PP pool ($k_1 \cdot a_1$) was shown to be 100 times greater than the flux of PO_4 from CP to PO_4 ($k_4 \cdot a_4$). Furthermore, the flux of P from XP to CP was shown to be slightly larger than that from CP to PO_4 (i.e. there was a slow loss of CP to biologically unreactive forms). To obtain a mass balance with this model, the back-flux of PO_4 from the plankton ($k_5 \cdot a_2$) was required to be nearly equivalent to $k_1 \cdot a_1$. The possibility that a_1 was still too high was suspected (Lean 1973b), but no data were available. It was concluded that the flow of P via the XP and CP pools was small, but to balance uptake, a large back-flux of PO_4 from the PP pool into PO_4 ($k_5 \cdot a_2$) was necessary.

Lean's revision of the P exchange model suggested that uptake and back-flux were the dominant exchanges in Fig. 2. Therefore, turnover time of the PP pool due to backflux could still be estimated using eq. (2) and the lower estimate of activity in PO_4 based on filtration chromatography. While not emphasized in Lean (1973a, 1973b), the lower level of activity in PO_4 changed the calculated turnover time of the particulate fraction from 38 min in the above example to 380 min (0.26 d). This illustrates the sensitivity of the calculation to errors in estimating f_1 (fraction of ^{32}P in PO_4 at steady state).

Although the model in Fig. 2 is widely accepted, there is some evidence that large back-fluxes of PO_4 from P-starved plankton do not occur. Lean and Cuhel (1987) measured ^{32}P labelling of subcellular fractions of the PP pool during periods of rapid PO_4 turnover. Although uptake of external $[^{32}P]PO_4$ was in steady state in less than 1 h, subcellular movement of isotope continued for 12–18 h. Furthermore, pulse-chase experiments using additions of 1–5 μM PO_4 failed to cause any decline in any of the labelled particulate compartments. Instead, further increases in the isotopic labelling of the subcellular fractions simply stopped due to isotope dilution. These measurements indicated that the PP was not a homogeneous compartment and that the back-flux from the PP compartment was not detectable. If PO_4 exchanged rapidly between the particulate and dissolved forms, pulse-chase experiments should have resulted in a decline in activity in the PP pool.

These results suggest an alternative model of $[^{32}P]PO_4$ dynamics in lake water. Because the pulse-chase experiments of Lean and Cuhel (1987) did not reveal a change in activity in the PP pool, we hypothesized that the back-flux (k_5) had been overestimated. Furthermore, because chromatography had revealed that the activity in filtrate was approximately 90% organic P (XP + CP), we further hypothesized that filtration artifacts might be responsible for at least some of the observed activity in filtrate at steady state. Specifically, if delicate organisms were damaged during filtration, labelled and originally cellular organic P and PO_4 would appear in the filtrate. The release of intracellular $[^{32}P]PO_4$ during filtration would obscure the true pattern of $[^{32}P]PO_4$ incorporation into cells, particularly at the end of an incubation when soluble ^{32}P was a small fraction of the total activity. This would mean that both the size of the PO_4 pool (a_1) and the magnitude of the uptake flux ($k_1 \cdot a_1$) were also overestimated. If true, the magnitude of the back-flux term ($k_5 \cdot a_2$), needed for mass balance at steady state, would be similarly overestimated.

In the present investigation, dialysis membranes were used to test the hypothesis that cell damage during filtration could explain a portion of the observed steady-state distribution of isotope. This technique enabled us to estimate the amount of $[^{32}P]PO_4$ in solution without filtration during apparent isotopic steady state when current models indicated that a large back-flux of $[^{32}P]PO_4$ from the primary consumers of PO_4 had to occur to maintain the high levels of $[^{32}P]PO_4$ found after filtration. We used primarily 0.2-μm filters to produce filtrate, but the filtrate from other filters was also compared.

Methods

Experiments were conducted at Jack's Lake, on the edge of the Canadian Shield in Ontario (44°41′20″N, 78°02′54″W). The lake is located approximately 200 km northeast of Toronto and

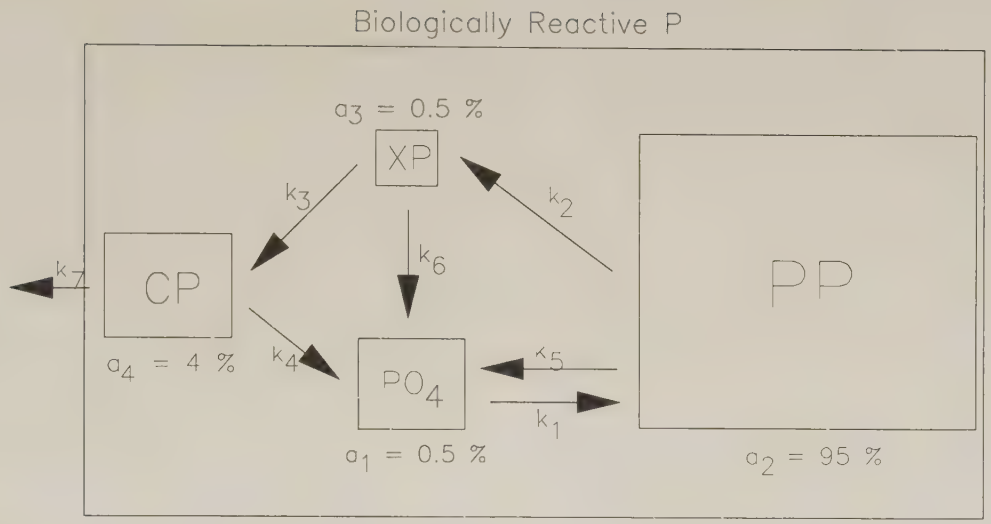

FIG. 2. Modified model of $[^{32}P]PO_4$ dynamics (Lean 1973a, 1973b). Terms are as in Fig. 1 and as follows: XP = unknown, small molecular weight, organic P; CP = colloidal, high molecular weight, organic P.

has been the site for other PO_4 studies (Taylor and Lean 1981, 1991; Lean and White 1983; Pick et al. 1984; Hamilton and Taylor 1987; Lean and Cuhel 1987). It has calcium concentrations of 14 mg/L, midway between softwater shield lakes and those in sedimentary drainage basins. Summer total P concentrations are near 12 μg P/L in surface waters with mean summer chlorophyll *a* values of 3.5 μg/L. Surface samples were taken from Sharpe's Bay, the largest section of the lake, during August and September 1989, and experiments were begun within 30 min.

Uptake kinetics for $[^{32}P]PO_4$ were conducted as described by Lean (1973b) except that 3 mL was filtered through 0.2-μm Nuclepore filters instead of 5 mL through 0.45-μm Millipore filters. Filtrate was collected directly in vials and counted by Cerenkov radiation in a Beckman model 1701 liquid scintillation counter. Gel permeation chromatography (Lean 1973a, 1973b) was conducted on filtrate using Sephadex G-25 fine resin packed to a depth of 35 cm in a 2.5-cm-diameter column. Samples (0.5–4.0 mL) were injected and eluted at a flow rate of 1.2 mL/min with a Buchler peristaltic pump using 0.2% sodium chloride and 0.02% sodium azide as a bacteriostatic agent. Sequential fractions (3 mL) were collected directly in vials using an LKB model 2112 fraction collector. Cerenkov counts were obtained as described above for filtrate. The column was calibrated with blue dextran and $[^{32}P]PO_4$ to determine the void volume for compounds >5000 molecular weight and the elution volume for the PO_4 peak, respectively.

Dialysis was performed using Spectra Por 7 membranes (50 000 molecular weight cutoff). Sections of tubing 2.2 cm in diameter by 7.5 cm in length were cut and soaked several times in NaCl/azide water to remove PO_4 contamination. Immediately prior to use, each tube was carefully clamped at one end with a tube closure supplied by the manufacturer. Without rinsing the interior, 10 mL of recently filtered (0.2 μm) lake water was introduced to the bag, air bubbles were removed, and the other end was clamped. The outside of the bag was rinsed briefly in filtered lake water and suspended in a stirred beaker of whole lake water at isotopic steady state (usually ~1 h after addition of isotope).

Time to 50% diffusion equilibrium in dialysis tubes was determined twice. Lengths (7.5 cm) of dialysis tube were placed in filtered lake water or NaCl/azide distilled water spiked with $[^{32}P]PO_4$. The dialysis tubes were sealed with a small piece of tygon tubing protruding for sampling. Over a 2- to 4-h period, aliquots were withdrawn for radioanalysis and compared with activities outside the tubes. Since dialysis is a slow process, limited by diffusion across the cellulose membrane, we selected conditions to achieve 50% of diffusion equilibrium in ~2 h. Tube length, internal volume, and stirring rate were the principal variables. Clearly, no resolution of temporal changes in ^{32}P distribution is possible with this approach. However, after isotopic steady state was achieved (as indicated by filtration), the method gave us an alternative estimate of dissolved $[^{32}P]PO_4$ activity without filtration. After 2 h of dialysis, the 10 mL within the dialysis tube was removed, subsamples were counted in duplicate by Cerenkov radiation to determine total activity of the dialysate, and a 4-mL subsample was injected onto the Sephadex column to separate molecular fractions as described above for filtered lake water.

Results

Movement of $[^{32}P]PO_4$ label into a dialysis bag from azide-treated distilled water reached 50% equilibration within ~2 h (Fig. 3). The mean ±SE for both calibrations was 114 ± 11 min, and dialysis bags were routinely left for 2 h in experimental samples which had reached apparent isotopic steady state. Activities so obtained were corrected to 100% of equilibrium values by multiplying by 2.

The dialysis bags adequately sampled the XP and PO_4 components of the dissolved fractions. When dialysis bags were placed in water samples without biologically active organisms present, i.e. azide-treated distilled water and filtered lake water, the distributions of label within the dissolved PO_4, XP, and CP pools remained within 10% of the initial values over a 4-h period (Fig. 3 and 4). This indicates that the dialysis bags themselves did not significantly influence the distribution of label or size of the P pools. Furthermore, the top panel of Fig. 4 illustrates that chromatographic analysis of dialysate from 2 to 4 h esti-

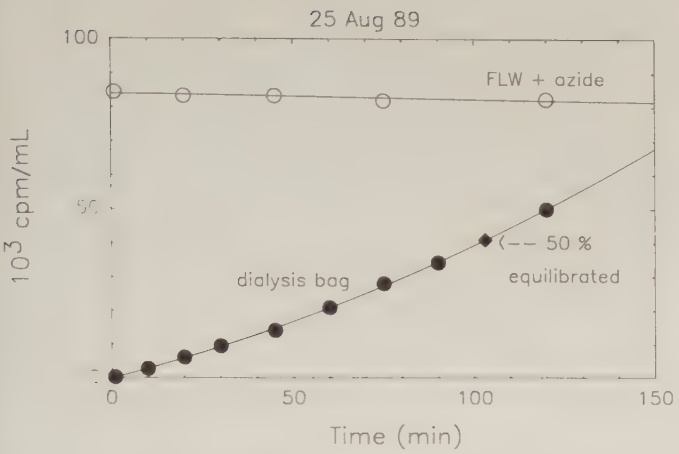

FIG. 3. Time course of diffusion of $[^{32}P]PO_4$ into a dialysis bag incubated in filtered lake water with 0.02% sodium azide. The diamond represents the computed 50% equilibration point at 103 min. FLW = filtered lake water.

mated the external $[^{32}P]PO_4$ as 97.3% of total activity whereas chromatographic analysis of filtrate at 0 and 2 h indicated 97.4 and 96.4%, respectively. XP estimates were also similar, although dialysis gave slightly lower activity than filtration. The 50 000 molecular weight cutoff of the dialysis membrane should not discriminate against XP (molecular weight ≈250), but underestimates of CP (molecular weight >5 000 000) are expected. The small differences shown in Fig. 4 are probably due to the small number of counts in the CP and XP fractions in these biologically immobilized calibrations. We did not specifically test easily measurable quantities of labelled XP as we did for PO_4 (Fig. 3). Originally, the term XP was used for a discrete peak eluting just prior to PO_4 (Lean 1973b), but here it is used for any activity between CP and PO_4.

Uptake of $[^{32}P]PO_4$ by surface plankton from Jack's Lake was rapid in August and September 1989. Turnover times for the dissolved pool were less than 2 min on four occasions, and an example is shown in Fig. 5. $[^{32}P]PO_4$ rapidly moved into the particulate fraction, with the percent in filtrate reaching an isotopic steady state after ~1 h. At this time, the filtrate contained about 6% of the total activity, and chromatography indicated that 3.6% of the total activity was in the PO_4 compartment (Fig. 6). Filtrate activities declined slowly over the next 3 h in a near steady state (Fig. 5), and dialysis was performed in the whole lake water during this period to provide an alternative estimate of the dissolved activity without filtration. As can be seen in Fig. 5, dialysis estimates without filtration (corrected to 100% equilibration) showed that only about 0.8% of the total activity was dissolved and 0.4% was $[^{32}P]PO_4$ (Fig. 6). Although CP is expected to be less in dialysate than in filtrate due to the molecular weight cutoff as described above, the estimate of $[^{32}P]PO_4$ in dialysate is expected to accurately reflect dissolved $[^{32}P]PO_4$ (Fig. 6). In this experiment, the dialysis estimate was about 1/10 of that in the filtrate.

All dialysis estimates of $[^{32}P]PO_4$ activity in whole lake water at isotopic steady state were lower than those in 0.2-μm filtrate (Table 1). The activity in 0.2-μm filtrate at apparent isotopic steady state was 2–6% of the total activity (second column of Table 1), and chromatographic estimates of $[^{32}P]PO_4$ in filtrate were 0.6–4% of the total activity (third column). However, dialysis of whole lake water indicated even lower values, 0.1–0.8% of the total activity in solution and 0.09–0.5% as

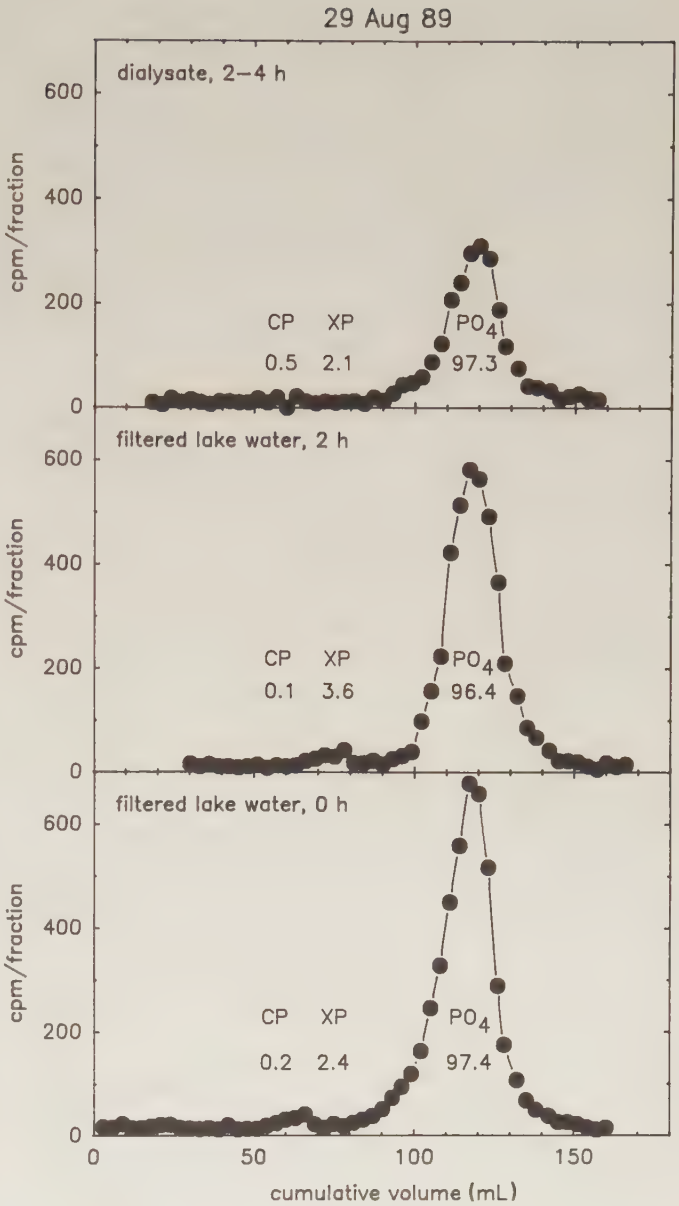

FIG. 4. Sephadex G-25 chromatography of $[^{32}P]PO_4$ added to 0.2-μm filtered lake water biologically immobilized with 0.02% azide. The lower two panels are chromatograms of the filtered lake water at time 0 and 2 h after isotope addition, and the uppermost panel is a chromatogram of dialysate obtained 2–4 h after isotope addition. The numbers are the percentage of the total activity represented by each pool ($f \cdot 100$). All chromatograms showed similar distributions and activities (the dialysate activity has not been corrected for 50% equilibration).

$[^{32}P]PO_4$. In general, dialysis estimates of $[^{32}P]PO_4$ in whole lake water were an order of magnitude (range 3–16) less than those estimated by 0.2-μm membrane filtration, supporting the hypothesis that filtration at isotopic steady state causes the release of intracellular $[^{32}P]PO_4$ and creates filter-passing ^{32}P-labelled organic P.

Further support for the accuracy of dialysis estimates comes from the last two lines of Table 1. We filtered half of the whole lake water at isotopic steady state on 18 September 1989 and then added a dialysis bag to the filtrate, i.e., we estimated filtrate $[^{32}P]PO_4$ with dialysis techniques. Chromatographic estimates of $[^{32}P]PO_4$ directly from the filtrate and from the

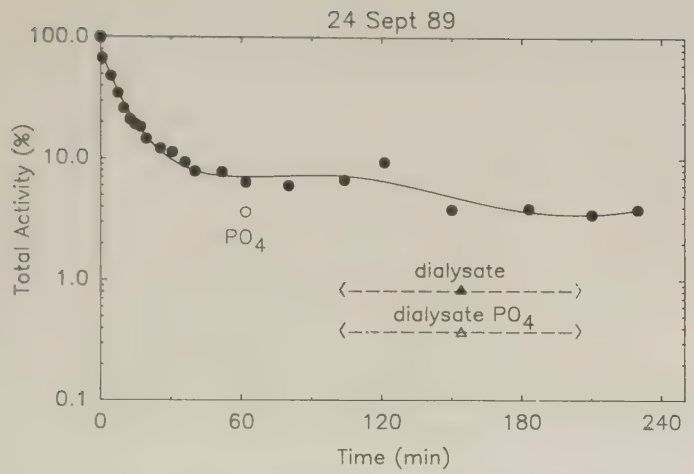

FIG. 5. Time course of ^{32}P distribution after $[^{32}P]PO_4$ was added to a whole lake water sample. Symbols: solid circles, filtrate activity; open circle, phosphate activity in filtrate determined by chromatography in Fig. 6; solid triangle, dialysate activity; open triangle, phosphate activity in dialysate. The horizontal arrows denote the length of the equilibration period for dialysate; all data are expressed as a percentage of total ^{32}P activity.

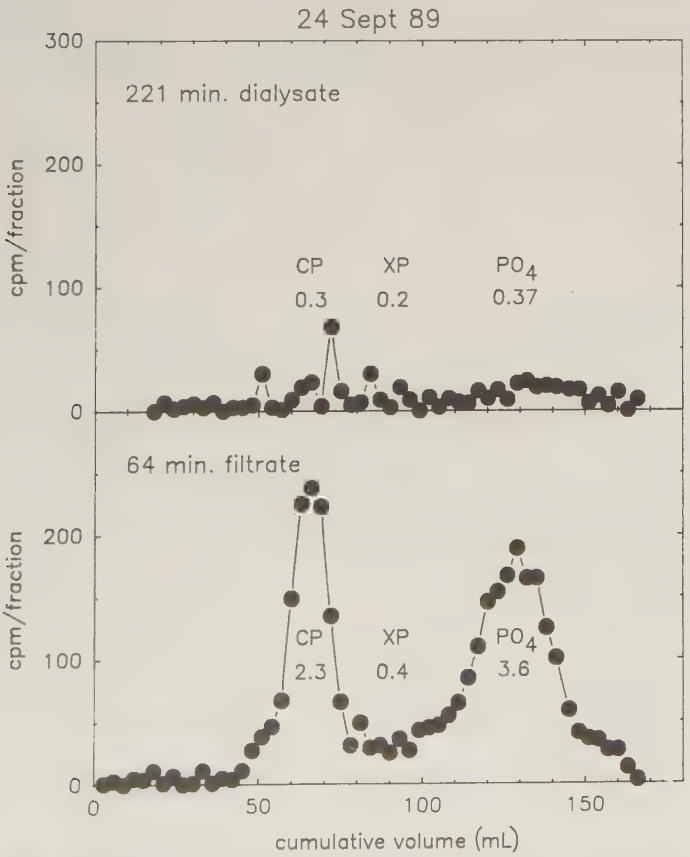

FIG. 6. Sephadex G-25 gel chromatography of filtrate and dialysate from the time course of $[^{32}P]PO_4$ uptake shown in Fig. 5.

dialysate agreed within 10–30%. In other words, when we deliberately created a filtration artifact by filtering first and then dialysing, we obtained approximately the same $[^{32}P]PO_4$ activity with both dialysis of filtrate and direct counting of filtrate. This indicates that dialysis accurately estimated the percentage of the total activity as $[^{32}P]PO_4$ without significant problems of

TABLE 1. Estimates of total activity and $[^{32}P]PO_4$ activity in filtrate of 0.2-μm membrane filters and dialysate of Spectra Por 7 membranes (50 000 molecular weight cutoff). Whole lake water was incubated with $[^{32}P]PO_4$, and dialysis was performed when filtrate indicated isotopic equilibrium, as in Fig. 5. Activity in the PO_4 pools determined by filtration + chromatography and dialysis + chromatography is shown as Filt. PO_4 and Dial. PO_4. Multiple entries under some dates indicate sequential chromatographic determinations of activities after isotopic equilibrium.

	% of total activity in:				Filt. PO_4
Date	Filtrate	Filt. PO_4	Dialysate	Dial. PO_4	dial. PO_4
			Whole lake water		
31 Aug.	2.9	1.4	0.27	0.088	16.0
	2.1	0.61	0.14	0.13	4.7
18 Sept.	2.6	1.4	0.77	0.50	2.8
24 Sept.	6.3	3.6	0.85	0.37	9.7
			Filtered lake water		
18 Sept.	2.6	1.4	2.1	1.9	0.7
			1.9	1.5	0.9

(e.g.) dialysis tube sorption. Even more importantly, when we estimated the percentage of the total activity as $[^{32}P]PO_4$ without filtration, estimates of dissolved $[^{32}P]PO_4$ activity from dialysis were three times lower (Table 1, data line 3).

Comparisons of filter retention provided further insights for interpretation of ^{32}P dynamics in lake plankton. On 31 August 1989, subsamples were taken from a whole lake water sample at isotopic steady state after 1–4 h of incubation with $[^{32}P]PO_4$. Filtration indicated that only 9% of the ^{32}P was in the >5 μm fraction, 22% was in particles between 5 and 1 μm, and, as typically found during periods of rapid turnover, 69% was between 1 and 0.2 μm (Table 2). Bacterioplankton were clearly important in phosphate uptake on this date. The results in Table 2 are ranked by the % D column (percentage of total activity in the dissolved pool), and the ranking by % D virtually agreed with the stated pore sizes of the filters, indicating passage of small organisms through the larger pore sizes.

There is evidence for an artefact in the filtrate comparison of Table 2. The 0.2-μm filters allowed only 2% of the total ^{32}P activity to enter the filtrate compared with 4% for 0.45-μm Sartorius 11306 filters when <10 mL was filtered. However, when 50 mL was filtered through 4.5 cm-diameter, 0.45-μm Sartorius 11106 filters, as is routinely done for chemical analysis of filtrate P and soluble reactive P, the percentage passing the filter increased to 31%, and almost all of the filtrate activity was CP. The appearance of colloidal P (high molecular weight P) suggests that increasing filtration volumes caused cell fragmentation and passage through the filter. Furthermore, chromatography of dialysate yielded estimates of the percentage of total activity in $[^{32}P]PO_4$ 3–10 times lower than any filters (first data line of Table 2). This is consistent with the data shown above and our hypothesis of filtration artefacts and indicates that intracellular $[^{32}P]PO_4$ is also liberated during filtration of plankton at apparent isotopic steady state.

Cell damage during filtration is also demonstrated by a serial filtration study using the same water sample at apparent isotopic steady state. Sequential 3-mL aliquots of the water were filtered through the same 0.2-μm Nuclepore filter, and each 3-mL subsample of filtrate was counted separately (Fig. 7). The dis-

TABLE 2. Dissolved ^{32}P activity in dialysate and filtrate of various filters from a sample of whole lake water from Jack's Lake (Ontario) on 31 August 1989. Turnover time (based on 0.2-μm filtrate) was 15 min, and the dialysis and filtration comparisons reported below were begun after 60 min at apparent isotopic steady state. % D, % CP, % XP, and % PO_4 are the estimated percentages of the total activity in the dissolved, colloidal P, small molecular weight P, and orthophosphate pools. The latter three were determined by Sephadex G-25 chromatography of dialysate or filtrate. Dialysis data obtained at 50% equilibrium have been corrected to 100%.

Filter type	Filter pore size (μm)	Filtered volume (mL)	Dissolved or filtrate			
			% D	% CP	% XP	% PO_4
Dialysis membrane	—	—	0.27	0.057	0.097	0.12
Nuclepore	0.2	4	2.3	1.1	0.021	1.1
	0.2	9	2.1	1.3	0.18	0.63
Sartorius 11306	0.45	3	4.2	—	—	—
Whatman GF/F	0.8	3	30.	—	—	—
Sartorius 11106	0.45	50	31.	30.	0.065	0.32
Nuclepore	1.0	3	69.	—	—	—
Nuclepore	5.0	3	91.	—	—	—

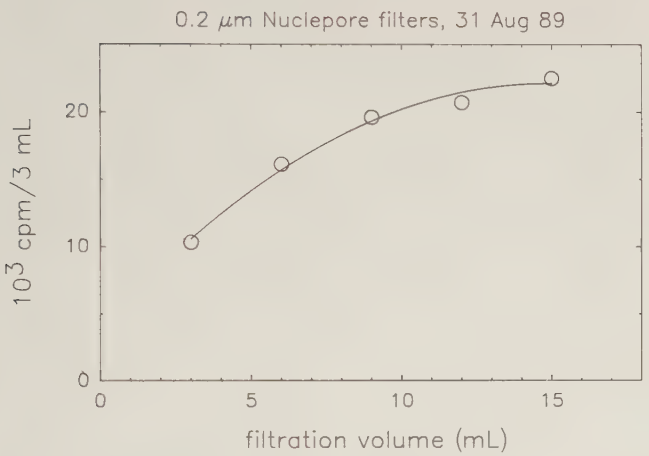

FIG. 7. Sequential filtration of 3-mL aliquots of water at apparent isotopic steady state through a single 0.2-μm Nuclepore filter.

solved activity in the filtrate more than doubled as the cumulative volume increased from 3 to 15 mL, i.e. as more material accumulated on the filter.

Discussion

The data presented above show that signficant artefacts occur during filtration of samples. At apparent isotopic steady state, most of the $[^{32}P]PO_4$ is in the particulate fraction and cell damage will result in production of labelled cell fragments or dissolved substances which will pass through the filter. Filtration artefacts can inflate estimates of dissolved PO_4 activity by up to an order of magnitude at apparent isotopic steady state (Fig. 6; Tables 1 and 2). However, it is likely that the magnitude of the problem is considerably lower during the initial phases of an incubation when the particulates are only partially labelled, i.e. the estimates of the fractional rate constants should be much less influenced by this problem than the steady-state distribution.

A potential mechanism for the production of ^{32}P-labelled cell fragments is stripping of microfibrils from phytoplankton cells. Using gentle filtration, Leppard et al. (1977) observed losses of cell-surface microfibrils from phytoplankton. During incubations with $[^{32}P]PO_4$, microfibrils probably become labelled as PO_4 moves across the cell membrane and is incorporated into organic P pools. If microfibrils are stripped from cells during filtration, these fragments may contribute $[^{32}P]CP$, and cell sap may contribute XP and PO_4 to the filtrate. Although we have no evidence that this process occurred in our samples, these observations provide an explanation of our results (e.g. Fig. 7; Table 2). Also, recent reports of intact organisms passing 0.2- and 0.4-μm filters (Li 1990; Stockner et al. 1990) lend further evidence that filtrate counts are overestimated, especially at the end of an incubation under P-limited conditions when most of the counts are in the smallest organisms (e.g. Table 2).

This analysis identifies a need for improved methods for separating dissolved from particulate ^{32}P-labelled compartments. Dialysis is too slow to resolve rapid uptake and was used here only to provide a method of estimating $[^{32}P]PO_4$ activity without filtration. We suggest the investigation of alternative methods for separation of particulate from dissolved fractions or careful analysis of filtration artefacts (e.g. Tarapchak et al. 1982). We offer the above evidence to indicate the magnitude of the problem.

The results presented above show that the size of the dissolved P pools has been overestimated at steady state in the isotope model of Fig. 2. Consequently, the magnitude of PO_4 uptake, PO_4 back-flux, and turnover rate of the PP pool has also been overestimated in planktonic P dynamics. In contrast, PO_4 turnover times (T_1) are probably reliable. As suggested above, estimates of the turnover rate of the PO_4 pool are least likely to be affected by cell damage during filtration because the rate is measured at the beginning of an incubation when particulates are only partially labelled. However, it is now clear that overestimation of dissolved ^{32}P activity at apparent isotopic steady state is likely in filtrates. Taylor and Lean (1991) reached a similar conclusion in their attempts to balance a model of P dynamics using functional components of the plankton.

The turnover time of the PP pool (T_2) is also likely to be longer than estimated in the introduction. Using the two-compartment exchange model (eq. (2)), the PP turnover time was 38 min when turnover of the PO_4 pool was 2 min and 5% of the total activity remained at isotopic steady state. When we used Lean's (1973b) chromatographic estimates of dissolved $[^{32}P]PO_4$ of 0.5% in filtrate, we computed a PP turnover time

of 0.3 d. Now with dialysis estimates of dissolved $[^{32}P]PO_4$ in the range of 0.09–0.5% of total activity (Tables 1 and 2), PP turnover times are even longer (0.3–1.5 d). These longer estimates of PP turnover time are in agreement with those computed from radiotracer labelling patterns of subcellular components (Lean and Cuhel 1987) and from models of P distribution and fluxes between functional components of the plankton (Taylor and Lean 1991).

Since these data provide evidence that $[^{32}P]PO_4$ at apparent isotopic steady state has been overestimated by a factor of 3–16 (Tables 1 and 2), does a back-flux of PO_4 from primary PO_4 consumers exist at all for P-limited populations? The pulse-chase experiments of Lean and Cuhel (1987) failed to cause declines in the labelling of particulate compartments, but Lean and White (1983) found that radiotracer uptake of added PO_4 was greater than chemically determined values for PO_4 uptake by an amount equal to a computed back-flux. However the back-flux was of a nonlabelled form and could be due to excretion by organisms other than phytoplankton and bacteria. The work of Lean and Nalewajko (1976) and Nalewajko and Lean (1978) on axenic cultures of freshwater algae examined net changes in subcellular P compartments by redistribution of $[^{33}P]PO_4$ and then measured uptake using $[^{32}P]PO_4$. In every case, uptake exceeded net change, suggesting a back-flux. Further work should be done to clarify the magnitude of the back-flux term.

We conclude that a back-flux of PO_4 may occur from the primary PO_4 consumers under P-limited conditions, but that the rate has been overestimated by an order of magnitude due to filtration artifacts. Realistic models of P dynamics in lake waters will depend on the determination of the magnitude of the PO_4 back-flux from primary consumers relative to PO_4 excretion by grazers. This issue is unlikely to be resolved until better methods are available for reliable separation of dissolved and particulate P components.

Acknowledgements

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Season and Body Size Influence Movement of Hydropsychid Caddisfly Larvae (Trichoptera: Hydropsychidae)

B. L. Kerans[1]

Department of Zoology, The Ohio State University, Columbus, OH 43210, USA

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Movement patterns of fifth-instar *Hydropsyche slossonae* larvae were investigated in artificial streams. In the first choice experiment, larvae were released into a central area and their upstream or downstream crawling or drifting was monitored. A second experiment used the same design; however, fifth instars collected during spring and fall were divided into two, nonoverlapping, relative size-classes (large and small) to determine how these factors influenced movement parameters. In both experiments, larvae always drifted more frequently than they crawled, but in experiment 2, drifting tendency declined with increasing weight in spring. In both experiments, there was no statistically detectable directional bias to crawling nor change in relative crawling direction with season or size-class. In experiment 2, drifting larvae were lighter than nondrifting larvae in the large size-class, but equal in weight in the small size-class. This result was unrelated to absolute weight, as large size-class larvae in fall were similar in weight to small size-class larvae in spring. It is possible that the relative weight differential of drifting and nondrifting larvae in one size-class but not the other may be related to sexual rather than body size differences.

Les régimes de migration du cinquième stade larvaire de *Hydropsyche slossonae* ont été étudiés dans des cours d'eau artificiels. Au cours de la première expérience, on a surveillé le déplacement ou la dérive de larves, en aval ou en amont du point central de mise en liberté. La deuxième expérience était de conception semblable, sauf que les larves recueillies au printemps et à l'automne ont été divisées en deux groupes distincts de longueur relative (grosses larves et petites larves) afin de déterminer si cette variable influait sur les parmètres de déplacement. Au cours des deux expériences, les larves ont toujours le plus souvent dérivé qu'elles ne se sont déplacées, mais au cours de la deuxième expérience, la tendance à la dérive a diminué en fonction de l'augmentation du poids au printemps. Lors des deux expériences, aucune direction préférentielle statistiquement identifiable du déplacement n'a été relevée, ni aucune variation de cette direction en fonction de la saison ou du groupe de longueur. Dans la deuxième expérience, les larves dérivantes de grande longueur étaient moins lourdes que les larves non dérivantes du même groupe, mais elles étaient de même poids dans le cas du groupe de petite longueur. Ce résultat n'était pas lié au poids absolu étant donné que les larves d'automne de grand longueur étaient de poids semblable aux larves de printemps de petite longueur. Il est possible que la différence entre le poids relatif des larves dérivantes et non dérivantes d'un des groupes de longueur, mais non de l'autre, soit liée à des différences de nature sexuelle et non à des différences de longueur.

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Organisms inhabiting the benthos of streams disperse by drifting downstream in the water column (reviewed in Müller 1954; Waters 1972; Brittain and Eikeland 1988) or by crawling on the substrate (e.g. Elliott 1969, 1971a; Bird and Hynes 1981; Söderström 1987). In situ studies of movement and colonization suggest that drift generally dominates (e.g. Waters 1972). One ecological prediction of the dominance of drift over crawling is downstream displacement of benthic populations. The fact that upstream areas of streams are not depopulated through drift has led researchers to investigate the magnitude of potentially compensatory upstream crawling (e.g. Bishop and Hynes 1969; Elliott 1971a). Whether upstream crawling can be an effective compensatory mechanism for downstream drift depends, in part, on movement preferences — relative tendencies of larvae to drift or crawl. For most organisms, movement preferences have not been adequately addressed under controlled experimental conditions. Moreover, the abundance of benthic insects in the drift and the tendency of some species to move in particular directions may be seasonally and ontogenetically variable (e.g. Bishop and Hynes 1969). Consequently, the magnitude of compensatory movements may vary.

Hydropsychid caddisfly larvae frequently occur in the drift and have been shown to crawl on the substrate (Elliott 1967, 1968, 1971a; Bishop and Hynes 1969; Walton 1980; Bird and Hynes 1981; Bergey and Ward 1989). Using estimates of drift and benthic densities, Hemsworth and Brooker (1979) suggested that hydropsychid populations should exhibit downstream displacement; however, the relative tendency of larvae to crawl upstream or move downstream has not been quantified. Hydropsychids also exhibit seasonal changes in drift tendencies. In laboratory and field experiments, numbers of fifth-instar *Hydropsyche slossonae* (Trichoptera: Hydropsychidae) drifting were lower in spring than during fall and winter (Kerans 1989). Two ontogenetic factors may be related to declining drift tend-

[1]Present address: Institute for Environmental Studies, Engineering Annex FM-12, University of Washington, Seattle, WA 98195, USA.

ency in spring. First, spring is the major pupation and emergence time for *H. slossonae* (Mackay 1979) and fifth instars (the final larval instar) may drift less frequently prior to pupation. Second, fifth instars also increase dramatically in weight in spring (Mackay 1984) and larger individuals may drift less frequently than smaller ones.

In this paper, I present the results of two laboratory experiments assessing the movement preferences of fifth-instar *H. slossonae*. The objectives of these experiments were (1) to determine the relative tendency of larvae to drift or crawl and any directional bias of crawling and (2) to investigate how body size and season influence larval movement. In the first experiment, I gave fifth instars a choice of crawling upstream, crawling downstream, or drifting. In the second, two relative size-classes of fifth instars were given choices similar to those in the first experiment in both spring and fall trials. I also compared the weights of drifting and nondrifting larvae in both experiments.

Methods

General Methods

All experiments were conducted in three replicate artificial streams (length 117.7 cm, width 15 cm, depth 15.7 cm). Streams were flow-through in design. Upstream, water passed through two mesh baffles to reduce turbulence. At the downstream end, water flowed through another mesh baffle and a 200-μm drift net and was collected in a large holding tank positioned under streams. Water was recirculated through streams using electric pumps. A chilling unit placed in the holding tank maintained water temperature.

An experimental section (27 × 15 cm) was positioned about 20 cm from the upstream end of each tank. This section was subdivided into three contiguous subsections: a central subsection (CEN, 13.5 × 15 cm) and two 6.75 × 15 cm subsections, one upstream (UP) and one downstream (DOWN) of CEN. Gravel (<4 mm in diameter, the material used for larval retreat construction) and pebbles (≈5 cm in diameter) were placed in all subsections. Areas upstream and downstream of the experimental section contained no substrates. Because hydropsychids inhabit and defend fixed retreats, the amount of surface area available for establishment could influence movement (Reice 1980); therefore, pebble (attachment sites for retreats) surface area was held constant across trials (≈500 cm^2 in CEN, ≈250 cm^2 in UP and DOWN). To determine pebble surface area, individual rocks were wrapped in aluminum foil and the foil was weighed. Surface area of each rock was estimated using standard regressions relating foil weight to surface area (Reice 1980). Because periphyton on pebbles can influence hydropsychid movement (Kerans 1989), pebbles were placed in riffles of Macochee Creek, a third-order tributary of the Mad River, Logan County, Ohio (see Kerans 1989 for detailed description of study site), for 1 mo prior to use to allow algal colonization.

Fifth-instar *H. slossonae* were collected from Macochee Creek. Larvae were fed finely ground trout chow ad libitum and held in well-aerated tanks, between 12 and 15°C, for less than 3 d before a trial. An individual was used in only one trial. In all trials, larvae were introduced into CEN in still water. Plexiglas dividers prevented larvae from moving from CEN; those that moved off pebbles were returned. After 1 h, the dividers were removed and flow increased to 35 cm/s. Drifting larvae were captured in the downstream net. Experimental trials began between 14:00 and 15:00 EST and ended the following morning between 09:00 and 10:00 EST. This time period was chosen because it encompasses peak movement times of *H. slossonae* (Kerans 1989). A 12:12 h day–night photoperiod (similar to natural spring and fall photoperiods) was maintained.

In other similar studies, a few fifth instars drifted during the first 30 min after flow initiation, and then drift declined to near zero until several hours after sunset (Kerans 1989). Therefore, I counted the number of larvae that drifted during the first 30 min to ensure that larvae were not merely being washed from CEN. Number drifting during the first 30 min was low (<8% of initial number). In the second experiment, I also counted the number of larvae in CEN that had constructed retreats to assess if larvae were actually using pebbles for attachment. Seventy-seven percent of larvae remaining had built retreats.

In the upstream section, current velocity was measured at six locations about 1 cm above pebble surfaces with a Marsh McBirney current meter. Current velocity, water depth, and water temperature were recorded at the beginning and end of each trial. Conditions were relatively constant across trials (flow 34.3 ± 0.5 cm/s, $n = 24$; depth 7.9 ± 0.1 cm, $n = 26$; temperature 13.6 ± 0.2°C, $n = 26$; all values ± 1 SE).

Exerpiment 1: Mode and Directional Bias of Movement

Ten experiments were conducted between August 1987 and March 1988 using either 25 (six trials) or 100 (four trials) larvae. At the end of each trial, numbers of larvae captured in the drift net and remaining in CEN, UP, and DOWN were determined. During this experiment, high-density trials were done because I was unsure whether the number of larvae crawling into UP and DOWN would be adequate to detect differences in movement parameters when using only 25 larvae. The (ln + 1) transformation was used on larval counts to successfully homogenize variances. To determine prevalent movement mode, a paired *t*-test compared number crawling (ln[UP + DOWN + 1]) with number drifting (ln[DRIFT + 1]). To determine directional bias of crawling, a paired *t*-test compared numbers crawling upstream with numbers downstream (ln[UP + 1] with ln[DOWN + 1]). Hydropsychids are not effective swimmers, so those found in UP would have crawled there. I also assumed that larvae crawled rather than drifted into DOWN because this subsection was shorter than known mean drift distances of hydropsychids (flow ≈30 cm/s, Elliott 1971b, 6.75 m; Otto and Sjöström 1986, 1.75 m) and the probability that an individual could enter DOWN after drifting was low (0.01 using Elliott's exponential model with mean drift distance of 6.75 m). Because almost all drift occurs at night, drifting was rarely directly observed; however, in the few drifts seen during the day, larvae were never observed entering DOWN. It was also possible that larvae crawling into UP or DOWN subsequently drifted or crawled back into CEN in which case, estimates of crawling would be conservative. Thus, the number reported crawling is the minimum number crawling overnight.

During trials conducted in August, visual inspection suggested that drifting larvae were smaller than those that remained in CEN. To test quantitatively for these weight differences, I compared the wet weight of drifting larvae with those that remained in CEN in the last five trials. At the end of a trial, individuals from randomly chosen subsamples (10 larvae where possible) of drifting larvae and those that remained in subsec-

tions were weighed. Wet weights (nearest 0.1 mg) were determined after blotting excess water from the larvae. Mean weights for subsections and drift were determined for each trial. A ln transformation on mean weights was used to homogenize variances. A paired *t*-test was used to compare transformed mean weights of drifting larvae with those that remained in CEN (ln[mean wgt CEN] with ln[mean wgt DRIFT]). Body size comparisons were not done on larvae in UP and DOWN because the number moving into these areas was low; thus, mean weights could not be estimated accurately.

Experiment 2: Influence of Body Size and Season on Movement

A two-way factorial design was used to test the effects of (1) relative body size (large and small size-classes) and (2) season (fall, September–December 1987; spring, April–June 1988) on movement. On the day of each trial, collections of fifth instars were visually inspected and larvae were divided into small and large size-classes by apparent body weight. Movement in the two size-classes was tested in separate artificial streams using 25 larvae for each group (deemed an adequate number, based on results of the first experiment). Trials were conducted as in experiment 1; larvae were placed in CEN and at the end of a trial, I counted the number of larvae in each subsection and the drift. There were four replicates for each season/size-class combination.

Wet weights of larvae were determined as in experiment 1, but for a maximum of five larvae in any subsection or drift. Because only a subsample of individuals was weighed, mean weight (X_T) and standard deviation of weight (S_T) for each trial were estimated using w_i = average wet weight in subsection i, n_i = number of larvae in subsection i, and s_i^2 = variance in wet weights in subsection i where $i = 3$ subsections (DRIFT, CEN, UP + DOWN). Equations were

$$X_T = \left[\sum_{i=1}^{3} n_i(w_i)\right] \Big/ \left[\sum_{i=1}^{3} n_i\right]$$

$$S_T^2 = \left[\sum_{i=1}^{3} (n_i - 1)s_i^2\right] \Big/ \left[\left(\sum_{i=1}^{3} n_i\right) - i\right].$$

One-way ANOVAs (using each season/size-class combination as a treatment level) were used to determine if there were significant differences in weight ($\ln[X_T]$) and variation in weight (S_T) among treatments. Two-way ANOVAs were used to determine whether number drifting (ln[DRIFT + 1]), movement mode (ln[DRIFT + 1] − ln[UP + DOWN + 1]), or crawling direction (ln[UP + 1] − ln[DOWN + 1]) differed between seasons or size-classes. Paired *t*-tests were used to examine differences within effects shown to be significantly different in ANOVAs. For example, if ANOVA showed that crawling direction differed between seasons, then separate paired *t*-tests would be done in spring and fall to determine the crawling direction prevalent in that season.

Because actual weight varied within each season/size-class treatment combination, regression analysis was used to determine how percentage drifting changed with mean trial weight (X_T) in each season. A multiple regression technique, using X_T and indicator variables for each season as independent variables, allowed simultaneous fitting of separate regressions for each season and comparison of the slopes and intercepts of the regression lines (Neter et al. 1983).

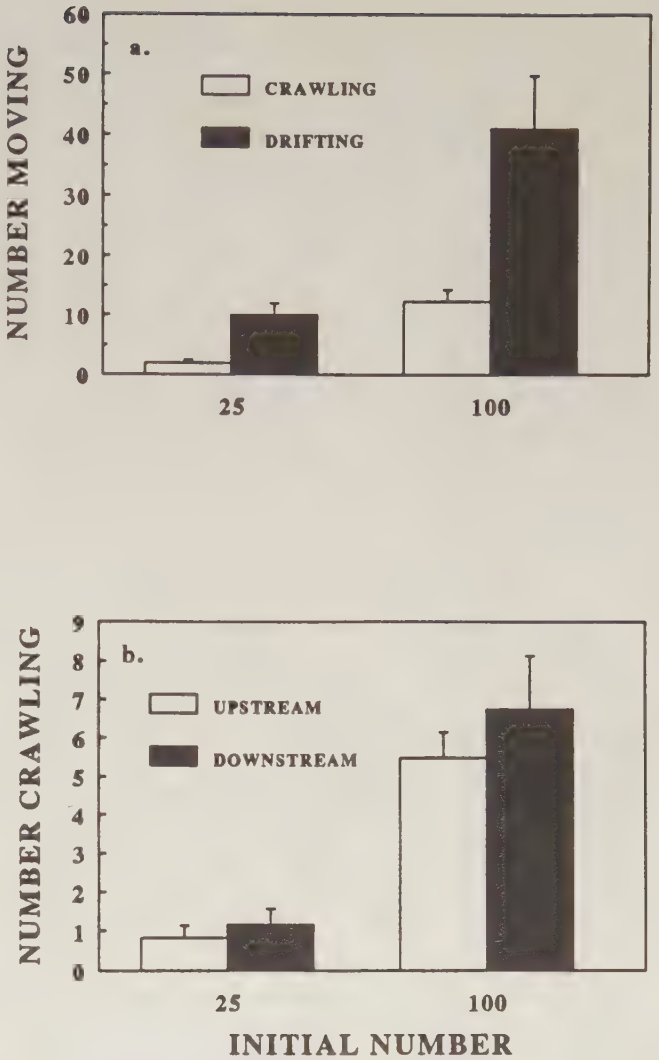

FIG. 1. Mean number of fifth-instar *H. slossonae* (a) crawling and drifting and (b) crawling upstream and downstream under low (n = 4) and high (n = 6) initial number of larvae. Error bars, ±1 SE.

TABLE 1. Averages of weights of fifth-instar *H. slossonae* larvae (X_T) and of the standard deviations of weight (S_T) of the four trials in each treatment combination. Averages (±1 SE) connected by lines are not significantly different (Tukey's honest significant difference test, α = 0.025 (Bonferroni adjustment for two comparisons)).

	Small/fall	Small/spring	Large/fall	Large/spring
Average X_T (mg)	8.9 (0.5)	11.5 (1.1)	14.2 (0.9)	24.1 (2.7)
Average S_T (mg)	1.8 (0.4)	2.1 (0.5)	2.3 (0.4)	5.0 (0.5)

Two-way ANOVA was used to determine if relative weight of drifters and nondrifters (ln[mean wgt CEN] − ln[mean wgt DRIFT]) changed with season and size-class. Paired *t*-tests determined whether drifting or nondrifting larvae were heavier within effects shown to be different in ANOVAs.

Results

Experiment 1

Drift was the predominant mode of movement (Fig. 1a). This pattern was similar across densities, although it was significant

TABLE 2. Two-way ANOVAs for numbers of fifth-instar *H. slossonae* larvae drifting, drifting and crawling (number drifting/number crawling), and crawling direction (number upstream/number downstream) in two seasons (fall and spring) and relative size-classes (large and small). In ANOVAs, critical $\alpha = 0.017$ (Bonferroni adjustment for three comparisons).

	Treatment	df	SS	*F*	*p*
Drift	Model	3	0.619	3.62	0.045
	Season	1	0.193	3.39	0.091
	Size-class	1	0.199	3.49	0.086
	Interaction	1	0.228	3.99	0.069
	Error	12	0.684		
Drifting vs. crawling	Model	3	2.96	1.32	0.313
	Season	1	1.28	1.72	0.215
	Size-class	1	0.63	0.84	0.378
	Interaction	1	1.05	1.41	0.258
	Error	12	11.93		
Crawling direction	Model	3	2.54	2.21	0.140
	Season	1	0.16	0.42	0.532
	Size-class	1	2.07	5.41	0.038
	Interaction	1	0.31	0.81	0.386
	Error	12	7.13		

only at low density ($t_5 = 3.95$, $p = 0.011$) and marginally nonsignificant at high density ($t_3 = 3.02$, $p = 0.057$). There was no directional bias to crawling, and the number of animals moving upstream was similar to the number that moved downstream (Fig. 1b, *t*-tests, $p > 0.275$ in each test). Nondrifting larvae (17.28 ± 0.89 mg (1 SE), $n = 5$) were heavier than drifting larvae (15.00 ± 0.36 mg (1 SE), $n = 5$; $t_4 = 3.24$, $p = 0.032$).

Experiment 2

Larval weight varied across seasons and size-classes (Table 1, ANOVA, $F_{3,12} = 23.63$, $p < 0.0001$). There was no overlap in larval weights in the two size-classes in either season (Table 1). Larvae were heaviest in the spring/large size-class and lightest in the fall/small size-class, while the spring/small and fall/large size-classes overlapped in weight (Table 1). Weight variation (S_T) was similar in all size-class and season combinations except in the spring/large size-class where variation was highest (Table 1, ANOVA, $F_{3,12} = 10.07$, $p = 0.0013$).

I was unable to detect any statistically significant change in relative drifting or crawling behaviour and in crawling direction with season or size-class (Table 2). Fifth instars always drifted more than they crawled (Fig. 2a, $t_{15} = 8.11$, $p < 0.0001$). There was no directional bias to crawling (Fig. 2b, $t_{15} = 0.28$, $p > 0.780$), although there was a suggestion that larvae in the large size-class crawled upstream more frequently than downstream (Table 2; Fig. 2b).

In the ANOVA, number of larvae drifting did not change with season or size-class (Table 2; Fig. 2a). However, in regressions, percentage drifting declined with increasing weight in spring (slope negative, $t_{12} = -3.41$, $p = 0.005$) and was unrelated to weight in fall (slope positive, $t_{12} = 0.35$, $p = 0.735$) (Fig. 3). The two regressions were statistically indistinguishable (neither slopes ($t_{12} = -1.61$, $p = 0.133$) nor intercepts ($t_{12} = 1.50$, $p = 0.160$) differed).

The relative weight of drifting and nondrifting animals differed between size-classes (ANOVA, $F_{1,12} = 9.78$, $p = 0.009$) but not between seasons (ANOVA, $F_{1,12} = 0.00$, $p = 0.966$)

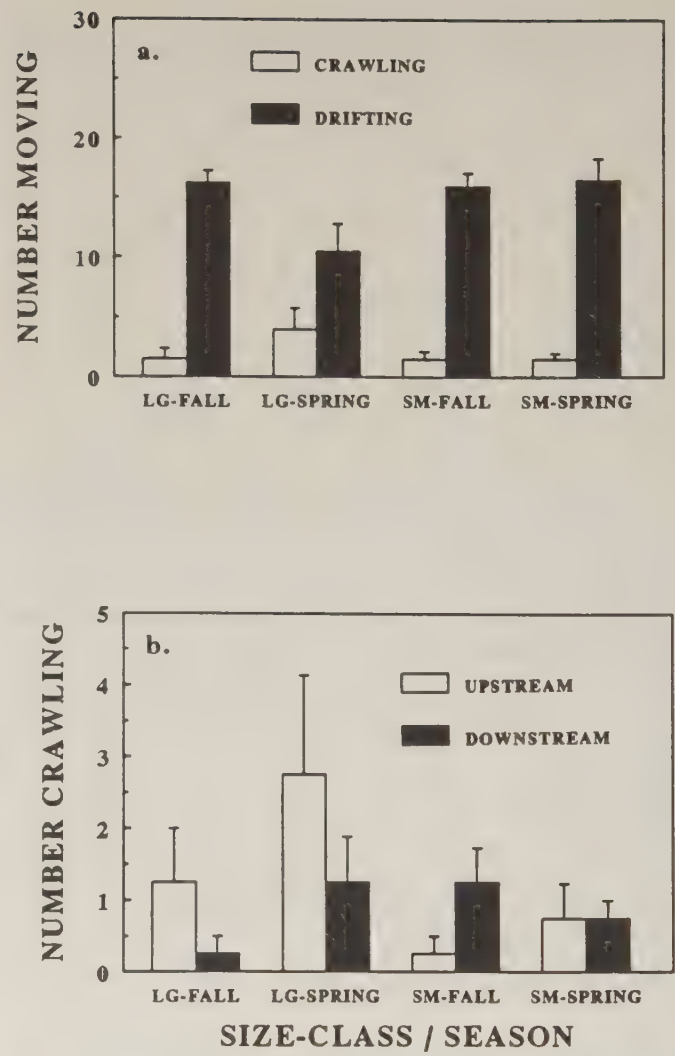

FIG. 2. Mean number of fifth instar *H. slossonae* (a) crawling and drifting and (b) crawling upstream and downstream for large and small size-classes in fall and spring ($n = 4$ in each treatment combination). Error bars, ±1 SE.

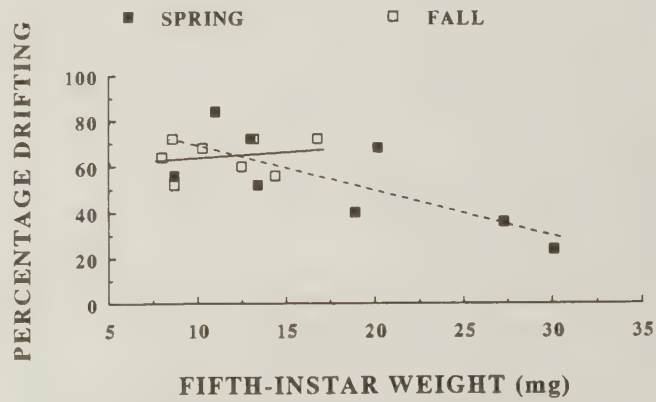

FIG. 3. Percentage of fifth-instar *H. slossonae* drifting as a function of mean wet weight of larvae used during each trial (X_T). Multiple regression was used to simultaneously fit lines in fall and spring; overall $r^2 = 0.56$, $F_{3,12} = 4.99$, $p = 0.018$, $n = 16$. Individual regressions: $y = 58.88 + 0.49$ weight (fall, solid line); $y = 88.87 - 1.96$ weight (spring, broken line). Lines begin and end at extreme mean values (fall, 8.0–16.8 mg; spring, 8.7–30.0 mg).

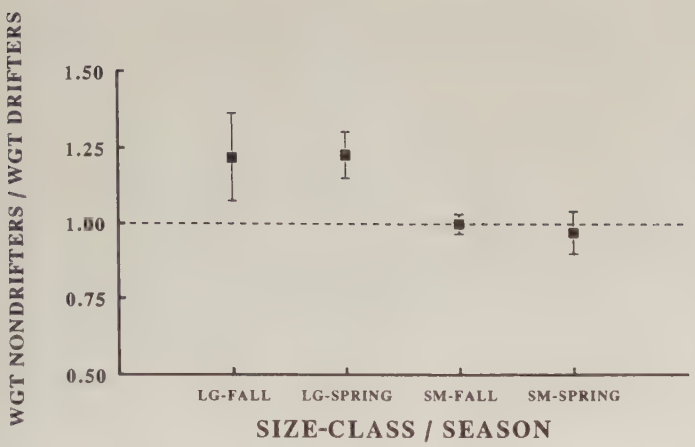

FIG. 4. Ratio of the mean weight of nondrifting larvae and mean weight of drifting larvae for large and small size-class *H. slossonae* fifth instars in spring and fall ($n = 4$ for each treatment combination). Error bars, ± 1 SE.

or between size-classes within seasons (ANOVA interaction, $F_{1,12} = 0.18$, $p = 0.678$). Nondrifting larvae were heavier than drifting larvae in the large size-class ($t_7 = 3.54$, $p < 0.005$) but not in the small size-class ($t_7 = 0.55$, $p > 0.250$) (Fig. 4).

Discussion

Fifth-instar *H. slossonae* drift more frequently than they crawl regardless of season or size-class. Crawling was infrequent, and only a maximum of 30% of larvae that moved exhibited this behaviour. The magnitude by which this experiment potentially underestimates crawling (if larvae crawled into UP and DOWN and subsequently drifted or crawled back into CEN) would depend directly on crawling rate. Elliott (1971a) found that *H. siltata* could crawl over 75 cm overnight. I observed *H. slossonae* crawling only in standing water; crawling ceased when flow was initiated. Although these observations suggest a low crawling rate for *H. slossonae*, they may not reflect nighttime activity. However, even if crawling was underestimated, because upstream and downstream crawling were equally probable and drift is, by definition, downstream movement, these data support the hypothesis that net dispersal direction of fifth instars is downstream.

Directional dispersal should result in downstream displacement of hydropsychid populations between reproductive periods when adult oviposition has the potential to redistribute populations. In most cases, by crawling upstream, fifth-instar *H. slossonae* were only able to compensate for about 10% of downstream movement (i.e. numbers crawling upstream were about 10% of total number moving downstream). Only in the large size-class in spring was upstream movement by crawling >20% of downstream movement. In in situ studies of colonization, upstream crawling is thought to generally compensate for <20% of downstream dispersal (Elliott 1968; Townsend and Hildrew 1976); however, Bergey and Ward (1989) suggested upstream compensation of 88%. Although quantitative comparisons of results across in situ studies are difficult because of differences in experimental designs, samplers, duration of experiments, and species, all suggest the same qualitative result — net downstream movement.

Comparisons between laboratory experiments of preference and field studies of colonization are difficult because behaviour is only one component of the colonization process. In both laboratory and field studies the number of larvae colonizing an area is influenced by the abundances of larvae in source areas and the probability of an individual immigrating to that site (Sheldon 1977; Minshall and Petersen 1985; Kerans 1989). Further, the probability of an immigrant reaching a particular site is related to the individual's preferred mode and direction of movement and the spatial relationship between source and "colonization" areas. In the laboratory, both the number of larvae in source areas and the spatial relationship between source and colonization areas are known.

Generally, with in situ studies, the spatial relationship between source and colonization areas and abundance of larvae in sources are unknown and probably vary because of the extreme spatial heterogeneity of benthic distributions. For instance, hydropsychid abundance is highest in riffles but they do enter pools (Walton 1980). Within a riffle, abundance also varies with current velocity (Kerans 1989; Schlosser and Ebel 1989). Brown and Brown (1984) even found hydropsychid densities to be highest in heads of riffles. Sources may also vary with movement mode. The source of drifting larvae captured at one point is cumulative from areas of varying distances upstream, while sources for crawling larvae are probably closer to the area being colonized and may include larvae that inhabit hyporheic zones.

Thus, laboratory preference and field colonization experiments measure different things. Colonization experiments measure the potential for a particular area to be recolonized from crawling or drift sources. On the other hand, laboratory choice experiments accurately quantify behaviour, but give only partial information concerning the in situ colonization process because they lack the full range of natural spatial heterogeneity. Results from laboratory experiments may only reflect preferences in the particular habitats used in tests. The type of study used will depend on the question of interest.

Body size and season interact to influence movement preferences for *H. slossonae* larvae. The number of larvae that drifted was reduced only as larvae increased in size in spring, and there was a suggestion that larger larvae also crawled upstream more frequently in spring. Size- and stage-specific variation in movement could indicate shifts in the ontogenetic niche (Werner and Gilliam 1984). For example, fifth instars of some hydropsychid species may move to new sites prior to pupation (Philipson 1953). Rutherford and Mackay (1985) showed that the vertical distribution of *H. slossonae* larvae and pupae in substrates differed and suggested that this may result because larvae move prior to pupation. Perhaps large *H. slossonae* show a decreased tendency to drift because they are crawling to find suitable pupation sites. Regardless of what factor causes these changes in movement, it appears to be intrinsic to larvae, rather than induced by environmental variation, because large and small larvae have different tendencies to drift during spring under the same experimental conditions. Niche shifts have the potential to create complex stage-, age-, or size-related interactions as individuals enter new habitats and potentially face different suites of predators, competitors, and physical conditions.

Interestingly in the large size-class, drifting larvae were always lighter than nondrifting larvae regardless of season and body weight. Hydropsychid drift has been shown to be density dependent (Kerans 1989), probably the outcome of aggresive interference (Glass and Bovbjerg 1969). Among the net-spinning caddisflies, aggressive encounters are often partially

mediated by body size — large larvae often "win" confrontations (Jansson and Vuoristo 1979; Hildrew and Townsend 1980; Otto 1989; Matczak and Mackay 1990). If heavier larvae cause lighter larvae to move away during an encounter, as occurs for other net-spinning caddisflies (Hildrew and Townsend 1980; Matczak and Mackay 1990) and damselflies (McPeek and Crowley 1987), then lighter, drifting larvae may be "losers" in contests for attachment sites. For fifth instars in the large size-class, drift behaviour therefore appears to be mediated by body size.

There were no differences in weight between drifting larvae and nondrifting larvae in the small size-class. Variation in relative weight was not directly related to absolute body size, as fall/large size-class larvae were similar in weight to spring/small size-class larvae. Thus, it appears that some other factor besides weight differs between size-classes. One possibility is that size-classes actually differ in sex. In *H. slossonae*, male pupae are lighter and adults emerge before heavier females (Mackay 1984). If this difference in body size is consistent throughout the fifth instar, then in both seasons the large size-class probably contained mostly females, while the small size-class contained mostly males. Other hydropsychids show sexual dimorphism in body size; larvae destined to be female are larger than those destined to be male (Hauer and Stanford 1981).

If the large and small size-classes actually reflect sexual differences among larvae, then males and females may adopt different movement and competitive strategies. Differences in behaviour between sexes are a common phenomenon in insects, often associated with the mating process and adults (e.g. Thornhill and Alcock 1983). Differences in behaviour of the sexes in larvae have been shown less frequently (Lederhouse et al. 1982). Although Trichopteran adults exhibit sexual differences in morphology and behaviour (e.g. Neves 1979; Thornhill and Alcock 1983), my observations may be the first suggestion of sexual differences in larval behaviour. Perhaps the behaviours (and sexual dimorphism) reflect variation in selection processes operating on males and females resulting from differences in reproductive tactics. Female larvae may be heavier because their reproductive capacity is limited by size and heavier individuals will gain selective advantages by producing more young (Slatkin 1984; Shine 1989). As trichopteran adults are short-lived (Wiggins 1984), energy for conversion into eggs must be obtained during the larval stage. By defending sites based on body size, large females will remain in areas where they were successful. If the small size-class is predominantly male, then males may use some other attribute to determine contest winners. Tests of these hypotheses will require determination of sex of the larvae and relationships with body size and behaviour.

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Usefulness of Meristic and Morphometric Characters in Discriminating Populations of American Shad (*Alosa sapidissima*) (Ostreichthyes:Clupeidae) Inhabiting a Marine Environment

Gary D. Melvin[1]

Department of Biology, University of New Brunswick, Fredericton, N.B. E3B 5A3, Canada

Michael J. Dadswell[2]

Biological Sciences Branch, Department of Fisheries and Oceans, St. Andrews, N.B. E0G 2X0, Canada

and Joseph A. McKenzie

Department of Biology, University of New Brunswick, Fredericton, N.B. E3B 5A3, Canada

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American shad (*Alosa sapidissima*) concentrate each summer in the basins of the inner Bay of Fundy during their coastal migration. Tag returns from 6124 marked shad indicated that these fish return to every important shad spawning stream on the Atlantic coast. Ten meristic and 16 morphometric characters of shad collected from 14 rivers (range: Florida to Quebec) were used to develop linear discriminant functions (LDF). Variables which differed significantly ($p < 0.05$) between the sexes, year of sampling, and the time of collection during the spawning run were removed prior to LDF classification. The highest mean percent correct classification was achieved by the allocation of fish into regional groups, after classification into individual rivers, using pooled meristic and morphometric variables: 87.2% for males and 82.4% for females. The functions were then used to assign shad from a mixed populations (Cumberland Basin) to individual rivers and regions. Of these, 10.9% were assigned to rivers south of Cape Lookout, North Carolina, 38.1% to rivers between Cape Lookout and Cape Cod, 32.5% into rivers of the Bay of Fundy, and 18.5% into rivers in the Gulf of St. Lawrence. In-river tag returns were compared with LDF classification and were found to be similar.

Lors de sa migration côtière, l'alose savoureuse (*Alosa sapidissima*) se regroupe en bancs dans les bassins du fond de la baie de Fundy. Les étiquettes récupérées de 6 124 aloses révèlent que l'espèce retourne à tous les importants cours d'eau natals de la côte de l'Atlantique. Les auteurs ont utilisé 10 caractéristiques méristiques et 16 caractéristiques morphométriques d'individus recueillis dans 14 rivières, à partir du Québec jusqu'en Floride, pour mettre au point des fonctions discriminantes linéaires (FDL). Les variables nettement différentes entre les sexes ($p < 0,05$), l'année d'échantillonnage et la période d'échantillonnage lors de la fraie ont été éliminées avant de réaliser les FDL. La répartition des aloses en groupes régionaux après classement selon les cours d'eau à l'aide des variables méristiques et morphométriques regroupées a donné le pourcentage moyen le plus élevé de classification exacte, soit 87,2 % dans le cas des mâles et 82,4 % dans le cas des femelles. Les auteurs ont ensuite utilisé les fonctions pour répartir les aloses d'une population mixte (le bassin Cumberland) entre des rivières et des régions. La répartition suivante a été obtenue : 10,9 % à des rivières au sud de Cape Lookout (Caroline du Nord), 38,1 % à des rivières entre Cape Lookout et Cape Cod, 32,5 % à des rivières tributaires de la baie de Fundy et 18,5 % à des rivières tributaires du golfe du Saint-Laurent. Une comparaison des données relevées d'étiquettes provenant d'aloses capturées dans ces bassins et de la classification par FDL a révélé que cette répartition était assez juste.

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Interest in the development of tidal power in the basins of the inner Bay of Fundy has prompted queries related to its possible impact on the environment (Gordon and Longhurst 1979; Dadswell et al. 1986). Of major concern are the effects development may have on migratory fishes that utilize these basins. Migratory species include the Atlantic herring (*Clupea harengus harengus*), alewife (*Alosa pseudoharengus*), blueback herring (*Alosa aestivalis*), American shad (*Alosa sapidissima*), Atlantic salmon (*Salmo salar*), striped bass (*Morone saxatilis*), Atlantic sturgeon (*Acipenser oxyrhynchus*), and the dogfish (*Squalus acanthias*) (Dadswell et al. 1984). Shad were selected for study as a representative of this migratory assemblage because of their size, abundance, relative ease of capture, wide geographical distribution, homing tendency (Hollis 1948; Nichols 1960; Melvin et al. 1986), and economic importance.

[1]Present address: Department of Fisheries and Oceans, Biological Station, St. Andrews, N.B. E0G 2X0, Canada.

[2]Present address: Department of Biology, Acadia University, Wolfville, N.S. B0P 1X0, Canada.

Each summer (June–September), large numbers of shad are known to inhabit the basins of the inner Bay of Fundy (Perley 1852; Leim 1924). This annual occurrence corresponds with the seasonal migratory behaviour of the species described by Talbot and Sykes (1958), Leggett and Whitney (1972), and Neves and Depres (1979). However, only recently has the northern limit been extended from the Gulf of Maine to include the basins of the inner Bay of Fundy (Dadswell et al. 1983, 1987). Shad tagged in the two major basins (Cumberland and Minas) of the inner Bay of Fundy have been recaptured in coastal and river waters from Labrador to the St. Johns River, Florida (Dadswell et al. 1987). The distributions of these fish serve to illustrate the potential scope of the impact of tidal power (Melvin et al. 1985; Dadswell et al. 1986).

Many investigators since Heincke's (1898) work concerning racial characters of Atlantic herring have used meristic and to a lesser extent morphometric characters to delineate races, populations, and/or stocks of fishes (Lund 1957; Raney and DeSylva 1953, 1957; Brown 1965; Williamson 1974; Merriman and Thorpe 1976; Messieh 1977; Lear and Sandeman 1980; Bowering and Misra 1982; Ni 1982). The practice of segregating groups, especially fishes, based on differences in vertebrae counts, lateral line scale counts, fin ray counts, and morphometric distances is well entrenched in systematics (Sneath and Sokal 1973) and fisheries biology (Royce 1964).

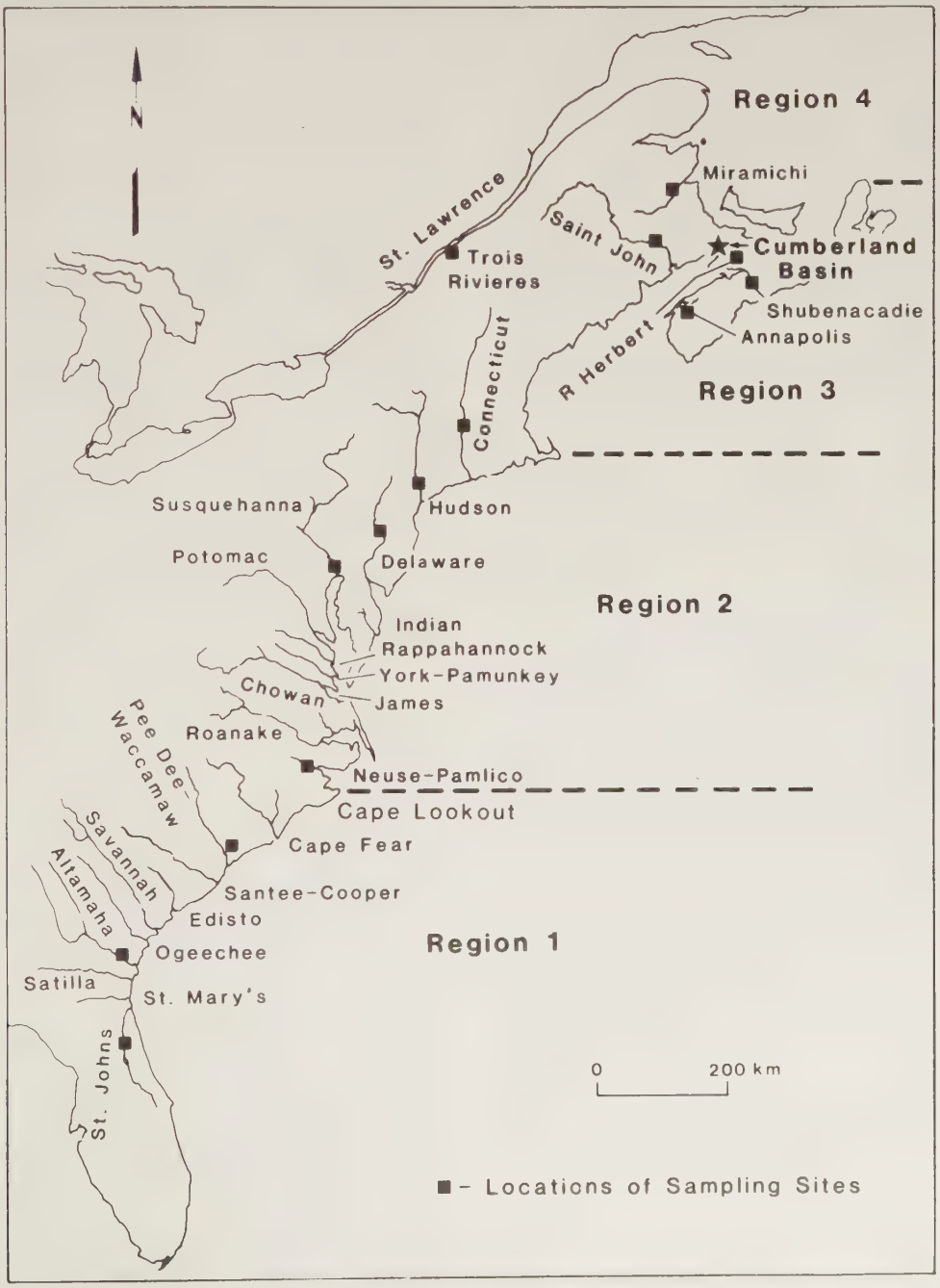

FIG. 1. Location of rivers sampled along the Altantic coast and the region divisions used in our discriminatory analysis.

TABLE 1. Number of American shad tagged and specimens collected, in parentheses, in Cumberland Basin by year and month.

Year	Month				
	June	July	August	September	Total
1979	153	256	43	42	494
	(53)	(46)	(0)	(42)	(141)
1980	698	1311	291	61	2361
	(72)	(131)	(101)	(11)	(315)
1981	341	2143	455	330	3269
	(50)	(97)	(40)	(22)	(209)
Total	1192	3710	789	433	6124
	(175)	(274)	(141)	(75)	(665)

Some authors have even described separate races solely on the basis of differences in a single character (Brown 1965).

The fact that an individual's processes and body form are susceptible to both genetic and environmental controls provides the foundation for variation in phenotypic expression. Slight changes in temperature, salinity, light, and dissolved gases during ontogeny (Taning 1952) can be reflected by significant differences in meristic counts for members of the same species. Morphological characters are subject to these factors for an even longer period (Martin 1949); consequently, the potential exists for even greater differences. Regardless of the cause, if these differences are significant and relatively constant with time, as are meristics (Hill 1959; Carscadden and Leggett 1975), they provide a useful means for identifying individuals from different populations.

The purpose of this study was to evaluate the usefulness of meristic and morphometric characters for discriminating fish from mixed populations of American shad in the ocean (i.e. Cumberland Basin). The approach was to quantify our ability to properly classify individuals and/or groups of individuals of known origin (river samples) using linear discriminant function (LDF) analysis. We assume that shad populations maintain differences because of the homing tendency of adults to natal streams (Hollis 1948; Carscadden and Leggett 1975; Melvin et al. 1986). The functions were then used to classify individuals of unknown origin (Cumberland Basin samples). The results of discriminant analysis were finally compared with tag return data for live shad tagged in the same marine site. Details of the marine study area, Cumberland Basin (Fig. 1), and the tagging program are presented in Dadswell et al. (1983, 1987) and Melvin (1984).

Materials and Methods

Operationally the collection of data for this study can be divided into two concurrent phases: Phase I, the tagging of shad to identify the spawning rivers to which fish returned and the collection of specimens for examination of meristic and morphometric variation in Cumberland Basin, and Phase II, the collection of fish from major shad spawning rivers along the Atlantic seaboard for analysis for inter- and intrariver differences of meristic–morphometric characters.

Phase I

American shad were sampled in Cumberland Basin between 1979 and 1981. Most weekdays, commencing the first of June and continuing to October 15, mixed gangs of five to seven gill

TABLE 2. Location, method of capture, date, and number of American shad collected from river systems of the Atlantic coast.

Location	Method of capture	Date			No. collected
		Year	Month	Day	
St. Johns R., Welaka, FL	Seine	80	02	27	41
Altamaha R., Jessop, GA	Gill net	80	02	29	17
Waccamaw R., Georgetown, SC	Gill net	82	03	03	44
Neuse R., New Bern, NC	Gill net	82	03	11	30
Susquehanna R., Havre de Grace, MD	Trap net	80	04	26	13
Delaware R., Lambertville, NJ	Seine	80	04	23	50
Delaware R., Lambertville, NJ	Seine	81	04	19	50
Hudson R., Verplanck, NY	Gill net	80	04	29	39
Connecticut R., Holyoke, MA	Fish lift	80	05	15	50
Annapolis R., U. Grandville, N.S.	Gill net	80	05	08	45
Annapolis R., Annapolis Royal, N.S.	Gill net	81	04	14	53
Annapolis R., Annapolis Royal, N.S.	Trap net	81	05	02	110
Annapolis R., Annapolis Royal, N.S.	Trap net	81	05	12	60
Shubenacadie R., Shubenacadie, N.S.	Gill net	80	05	07	51
Shubenacadie R., Stewiacke, N.S.	Gill net	81	05	19	34
Saint John R., Mactaquac, N.B.	Gill net	80	05	26	67
Miramichi R., Mill Bank, N.B.	Trap net	80	05	28	58
Miramichi R., Mill Bank, N.B.	Trap net	81	06	05	35
St. Lawrence R., Trois Rivières, Que.	Gill net	81	06	01	32

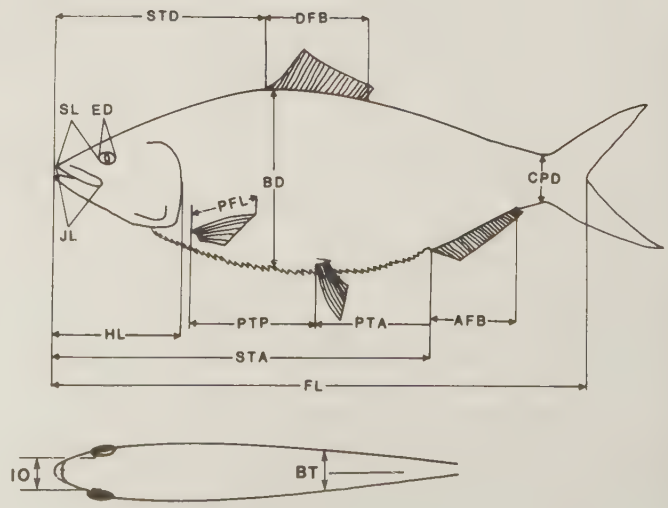

FIG. 2. Location of morphometric measurements made on each shad.

TABLE 3. Meristic and morphometric characters examined and acronyms used in this study.

Meristic		
1	DFR	Dorsal fin rays
2	AFR	Anal fin rays
3	PFR	Pectoral fin rays
4	ANS	Anterior scutes
5	POS	Posterior scutes
6	TOT	Total scutes
7	LLS	Lateral line scales
8	DVS	Dorsal–ventral scales
9	VERT	Vertebrae
10	GR	Gill rakers
Morphometric		
11	FL	Fork length
12	BD	Body depth
13	BT	Body thickness
14	CPD	Caudal peduncle depth
15	HL	Head length
16	SL	Snouth length
17	ED	Eye diameter
18	IO	Interorbital width
19	JL	Upper jaw length
20	DFB	Dorsal fin base length
21	AFB	Anal fin base length
22	STD	Snout to dorsal length
23	STA	Snout to anal fin length
24	PL	Pectoral fin length
25	PTP	Pectoral to pelvic fin length
26	PTA	Pelvic to anal fin length

nets (500–700 m) were set from a 7-m open vessel and allowed to drift with the tide (Dadswell et al. 1983). Mesh size of the 100-m multifilament nets ranged from 5.5 to 13.7 cm (diamond stretch measure) with a depth of either 6 or 12.5 m.

Gill nets were usually set for a period of 2 h before fishing. However, during times when water temperature exceeded 17°C and/or large numbers of shad were present in the Basin, the nets were hauled after 1 h to reduce mortalities. Each live shad removed from the net was marked with an individually numbered FTI Floy dart tag and released at the site of capture. During the 3-yr study period, a total of 6124 marked shad were released (Table 1).

Whenever possible, weather and fish availability permitting, 50 shad were retained biweekly. The fish were measured for fork length, total length, body depth, and weight, a scale sample removed for ageing (Cating 1953; Judy 1961), and a numbered label inserted under the operculum. All fish were individually packed on ice until they could be frozen (−20°C).

Phase II

Adult shad were sampled from 14 rivers (Fig. 1) within its reproductive range (Florida–Quebec) using gill nets (10.4- to 12.7-cm mesh), trap or pound nets (8.9- to 10.3-cm mesh), and haul seines (8.9-cm mesh). The selectivity of the fishing gear is discussed in the section on representation of sample. In Canada, all fish were collected by experimental gear; however, in the United States the majority of specimens were procured from commercial catches (Table 2). Five rivers, the Delaware, Hudson, Annapolis, Shubenacadie, and Miramichi, were sampled in two successive years to investigate between-year variation. In addition, because each river could not be visited at exactly the same period during the spawning run, the 1981 Annapolis River sampling was partitioned to represent three intervals: early, peak, and late phases of the run. All shad were subjected to the same measurements and packing procedures employed for retained fish in Phase I.

Laboratory Methods

For each retained fish, 10 meristic and 16 morphometric characters (Fig. 2; Table 3) were enumerated following descriptions outlined by Hubbs and Lagler (1947) with two exceptions: lateral line scales (LLS) were counted from the shoulder girdle laterally to the last scale transcending the apparent lateral line, including scales on the caudal fin base, and gill raker counts (GR) included rakers on both arches. All distances, excluding fork length (FL), body depth (BD), snout to dorsal (STD), and snout to anal (STA), which were measured to the nearest 1 mm, were determined using Vernier calipers to the nearest 0.1 mm. Vertebrae number and verification of fin ray counts were determined from X-ray plates. The fish were then opened for identification of sex and gonadal development stage and otoliths excised for confirmation of ages.

Statistical analyses were run on the Department of Fisheries and Oceans, Biological Station, St. Andrews, HP 3000 computer using SPSS (Nie et al. 1975) for descriptive statistics and LDF, BMDP for LDF and cluster analysis, and author-compiled FORTRAN programs for the calculation of Mahalanobis generalized distance (D^2, T^2) and associated F-values following procedures outlined in Kramer (1972). The D^2 values were adjusted for differences in sample size between groups using Royce's (1964) formula.

Representativeness of Samples

Shad collected for this study were obtained by three different types of fishing equipment which may have introduced gear-related size selection bias into samples. Trap nets and seines of the mesh sizes employed for river collections are generally considered nonselective for adult shad and as such assumed to collect a representative sample of the fish present at the time of sampling. Gill nets, especially those used by commercial fishermen, are known to be selective for larger fish. This was an unavoidable situation but in many areas was the only feasible way to collect specimens. To overcome this problem, slightly smaller mesh gill nets (10.2 cm), which have been found to capture a much wider range of fish, were employed whenever possible. It was assumed that, with respect to size, the shad collected from the rivers provided a representative sample of fish present at the time of capture for the following reasons.

(1) Mean fork length's of shad collected from individual rivers were in general agreement with the published findings for the same rivers (Warfel and Olsen 1947; Hill 1959; Nichols 1966; Miller and Jorgenson 1973; Shoubridge and Leggett 1978).

(2) An increase in mean fork length was observed for fish taken by nets of the same mesh size with increasing latitude. If the gear was highly selective for a particular size class, then it would be expected that similar lengths should be obtained for each river sampled (Table 4; Fig. 3).

(3) Comparison of the April 14 Annapolis River samples, collected from a 10.2-cm-mesh gill net, with May 2 and May 12 samples, taken from a 8.7-cm trap net, using a Student t-test indicated no significant ($p < 0.01$) difference in mean fork length for both sexes.

TABLE 4. Descriptive statistics of fork length for male and female shad by river of collection.

River	Male					Female				
	No.	Mean	Variance	Min.	Max.	No.	Mean	Variance	Min.	Max.
St. Johns, FL	24	37.7	5.7	13.4	41.3	17	40.4	8.9	33.9	45.0
Altamaha, GA	6	41.7	11.5	37.8	47.6	11	45.0	2.4	42.4	46.9
Waccamaw, SC	28	41.7	6.1	37.6	47.2	16	47.6	2.8	44.5	50.6
Neuse, NC	18	42.1	3.4	38.1	44.4	12	48.1	8.9	43.3	53.2
Susquehanna, MD	7	40.9	1.7	39.4	43.0	6	43.7	3.3	41.7	46.5
Delaware, NJ	57	42.4	10.7	28.9	48.0	43	49.2	5.4	42.8	53.4
Hudson, NY	21	44.7	13.3	37.3	50.0	68	47.1	8.2	39.0	52.2
Connecticut, MA	26	41.6	4.9	36.3	45.2	24	47.4	4.6	42.3	50.7
Annapolis, N.S.	145	45.8	12.4	38.3	57.2	133	49.7	18.9	42.4	60.3
Shubenacadie, N.S.	31	45.3	11.5	39.3	52.4	54	47.7	13.2	42.0	56.0
River Hebert, N.S.	23	46.0	5.4	41.9	50.0	21	48.8	5.3	45.3	52.4
Saint John, N.B.	25	42.8	11.9	36.7	49.5	43	47.3	17.9	40.1	56.5
Miramichi, N.B.	50	46.9	14.5	36.1	54.3	43	49.8	8.3	44.8	56.5
St. Lawrence, Que.	22	50.7	8.9	46.8	57.1	10	54.2	10.0	50.4	59.2

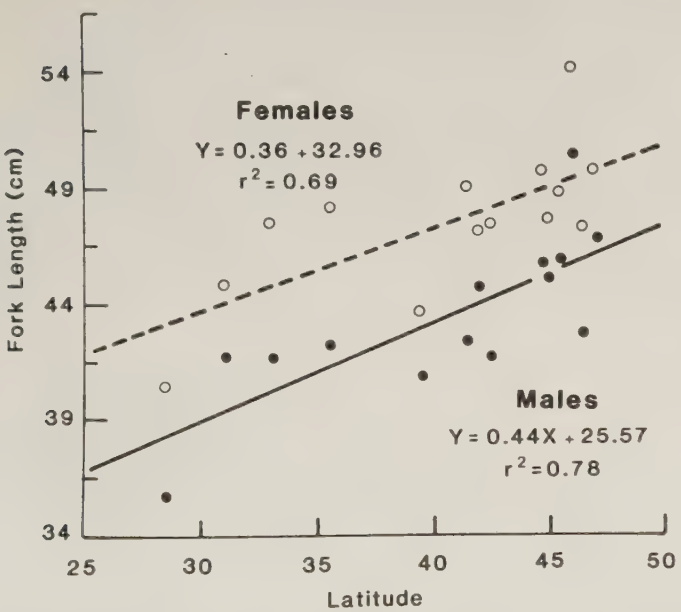

FIG. 3. Mean fork length of shad samples collected from 14 spawning rivers in relation to approximate latitude of the spawning site in the river.

Results

Variables were partitioned according to type (i.e. meristic or morphometric) for comparative purposes prior to pooling. Regression analysis of mean values with approximate latitude of spawning indicates a significant ($p < 0.05$) positive cline or trend from south to north for all but dorsal fin ray counts (Fig. 4). Descriptive statistics of fork length for male and female by river of collection are presented in Table 4. Descriptive statistics for all other characters are presented in Melvin (1984).

Character Variation

An important consideration in this study was character bias introduced by differences between sex, year of collection, and time of sampling during the spawning run. Multivariate comparison (Hotelling T^2 analysis) of male versus female meristic and morphometric data from the Delaware, Hudson, Annapolis, Shubenacadie, and Miramichi rivers showed significant differences ($p < 0.01$) for 7 of the 10 paired tests (Table 5). As a result, all data were separated by sex and analyzed accordingly. This served to remove the major differences in size observed between males and females (Table 4).

Between-year differences for each sex from the five rivers sampled in each of 2 yr were investigated using the same statistical procedure. Simultaneous confidence intervals were then employed to identify variables contributing to the significance. Upon removal of male and female LLS and eye diameter (ED) and female BD, the T^2 values were reduced to nonsignificant levels (Table 6). No significant differences were noted between times of sampling during the spawning run, after removal of the characters found to differ between years for the Annapolis River fish.

From these findings it was concluded that the meristic and morphometric characters examined from each of the Delaware, Hudson, Annapolis, Shubenacadie, and Miramichi rivers, upon separation by sex and omission of the above variables, did not differ significantly ($p < 0.05$) between year sampled and/or the time of collection during the spawning run. It was assumed as a working hypothesis that these conditions hold true for the other rivers which were not sampled in this manner. All data collected from a river were thus pooled prior to LDF.

Regional Groups

The use of LDF analysis to classify individuals of unknown origin into a priori groups is contingent upon the assumption that the individuals being classified belong to one of the defined groups. Specifically, for this study, it would imply that all individuals from Cumberland Basin must originate from one of the 14 rivers sampled — an unrealistic assumption. As well, because insufficient tag return data were acquired for comparison on a river to river basis, some form of grouping was necessary.

There are natural boundaries and anthropogenic conditions which provide a logical basis for a geographic division. The Gulf of St. Lawrence is separated from the Bay of Fundy by Nova Scotia creating two relatively isolated bodies of water. Furthermore, from the Canada–U.S. border to Cape Cod, access to river spawning areas of shad was almost completely denied through the construction of dams during the early part of this century (Walburg and Nichols 1967). This resulted in

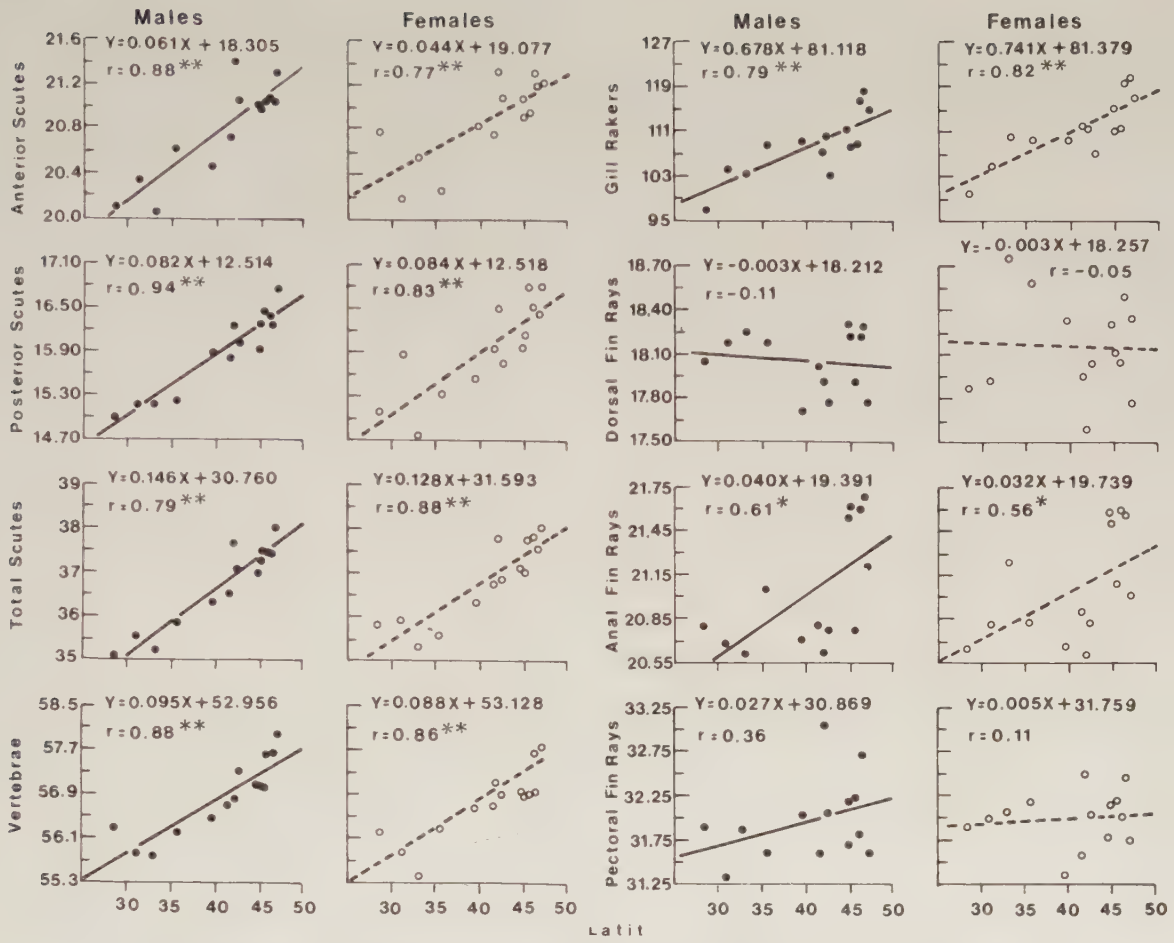

FIG. 4. Selected male and female meristic counts in relation to approximate latitude of spawning.

TABLE 5. Hotelling T^2 comparison of male versus female meristic and morphometric characters for the Delaware, Hudson, Annapolis, Shubenacadie, and Miramichi rivers, $^{**}p < 0.01$.

River	No. of fish	Meristic characters			Morphometric characters		
		D^2	T^2	F	D^2	T^2	F
Delaware	100	1.85	44.92	4.04**	53.91	1276.50	67.57**
Hudson	89	1.12	17.85	1.60	7.34	117.39	6.07**
Annapolis	278	0.94	64.69	6.27**	11.75	810.58	49.91**
Shubenacadie	85	0.79	14.92	1.33	5.49	104.31	5.34**
Miramichi	93	0.49	11.15	1.01	6.42	147.69	7.71**

the virtual annihilation of native shad spawning populations between the two points (Bigelow and Welsh 1925; Taylor 1951; Mansueti and Koeb 1953), creating a null zone between the Bay of Fundy and Cape Cod during the period of our study. The question which subsequently arises is whether another division is warranted south of Cape Cod and if so, where.

The reproductive strategies of shad spawning in the southern river of Florida, Georgia, and South and North Carolina have been described as distinctly different from those of rivers north of Cape Hatteras (Leggett and Carscadden 1978; Glebe and Leggett 1981), implying another division may be warranted. Based on reproductive biology and paired–grouped cluster analysis, another division which consisted of rivers south of Cape Lookout was established (Melvin 1984).

The shad's reproductive range was subsequently divided into four regions: Region 1, all rivers between and including Florida and Cape Lookout, represented by the St. Johns River (Florida), the Altamaha River (Georgia), and the Waccamaw River (South Carolina); Region 2, Cape Lookout to Cape Cod, represented by the Neuse River (North Carolina), the Susquehanna River (Maryland), the Delaware River (Delaware), the Hudson River (New York), and the Connecticut River (Connecticut); Region 3, rivers of the Bay of Fundy, represented by the Annapolis River (Nova Scotia), the River Hebert (Nova Scotia), the Shubenacadie River (Nova Scotia), and the Saint John River (New Brunswick); and Region 4, rivers of the Gulf of St. Lawrence represented by the Miramichi River (New Brunswick) and the St. Lawrence River (Quebec) (Fig. 1).

LDF: River Samples

LDF's were developed for each of 14 rivers from 9 meristic variables (character type 1), 14 female and 15 male morpho-

TABLE 6. Results of Hotelling T^2 statistical analysis of 10 meristic and 16 morphometric variables for 1980 versus 1981 samples by sex. The "before" values refer to significance level using all variables. Simultaneous confidence intervals were used to reduce F-values to nonsignificant levels. Variables removed were male and female lateral line scales and eye diameter and female body depth. $*p < 0.05$; $**p < 0.01$.

River	No. of fish	Meristic variables						Morphometric variables					
		Before			After			Before			After		
		D^2	T^2	F	D^2	T^2	F	D^2	T^2	F	D^2	T^2	F
Male													
Delaware	57	2.39	33.50	2.80*	1.44	20.12	1.91	6.47	90.78	4.12**	2.45	34.41	1.71
Hudson	21	11.16	33.49	1.76	4.38	13.14	0.85	2.89	8.68	0.11	9.00	26.98	0.47
Annapolis	145	0.74	8.84	0.83	0.69	8.23	0.87	4.63	55.53	3.13**	2.09	25.09	1.52
Shubenacadie	32	3.27	22.92	1.58	2.72	19.01	1.53	3.82	26.71	0.81	1.54	10.80	0.37
Miramichi	50	2.78	25.00	2.03	1.08	9.76	0.90	3.53	31.80	1.37	1.89	17.00	0.80
Female													
Delaware	43	2.84	28.39	2.22**	1.99	19.30	1.78	15.26	152.26	6.04**	3.21	32.05	1.56
Hudson	68	2.54	44.24	3.74**	0.77	12.30	1.23	8.62	146.56	7.08**	1.86	31.57	1.81
Annapolis	133	0.31	7.11	0.66	0.16	3.74	0.39	1.74	40.00	2.22**	0.71	16.31	1.05
Shubenacadie	54	1.97	23.66	1.95	1.42	17.01	1.59	5.03	60.39	2.69**	1.02	12.21	0.65
Miramichi	43	4.42	44.22	3.45**	1.11	11.27	1.10	5.86	58.64	2.32**	3.73	37.33	1.82

TABLE 7. Summary of the mean percent correct classification of character types by sex for raw, grouped, and transformed data as computed by LDF. The values in parentheses are the number of variables.

Data analyzed	Character type	Mean % correct classification		Minimum % correct classification		Maximum % correct classification	
		Male	Female	Male	Female	Male	Female
1. Raw river data	1	38.5 (9)	35.6 (9)	17.4	12.5	75.0	66.7
	2	59.0 (15)	53.1 (14)	42.1	38.2	100.0	100.0
	3	73.7 (24)	66.6 (23)	59.6	52.3	100.0	100.0
2. Log river data	1	38.7 (9)	34.5 (9)	8.7	12.5	75.0	70.0
	2	57.6 (15)	53.3 (14)	36.8	41.2	85.7	100.0
	3	73.3 (24)	66.1 (23)	59.6	53.3	100.0	100.0
3. Stepwise LDF river data (Wilks)	1	38.9 (8)	35.6 (8)	17.4	12.5	75.0	66.7
	2	60.5 (13)	53.3 (14)	43.9	38.2	100.0	100.0
	3	73.9 (20)	66.6 (21)	59.6	53.5	100.0	95.2
4. Regional groups	1	61.2 (9)	55.2 (9)	52.7	48.0	87.9	90.9
	2	74.4 (15)	74.3 (14)	67.6	68.6	79.3	84.1
	3	82.6 (24)	79.6 (23)	79.8	76.4	86.2	90.0
5. Log regional groups	1	61.8 (9)	55.7 (9)	52.7	46.3	87.9	92.0
	2	73.9 (15)	73.7 (14)	66.7	71.9	79.2	81.1
	3	82.4 (24)	78.4 (23)	79.8	76.1	88.9	93.2
6. Grouped by region after (3) above	1	61.8 (8)	57.0 (8)	51.8	52.0	84.5	79.5
	2	79.9 (13)	76.3 (14)	75.9	71.9	80.8	81.8
	3	87.2 (20)	82.4 (21)	81.0	79.4	89.2	86.4

metric variables (character type 2), and both variable types pooled together (character type 3), using the pooled covariance matrix. Separate analyses for the three variable types were conducted for comparison on both the raw and $\log_{10}$-transformed data. The data were subjected to stepwise LDF (Wilks lambda) to investigate the effect of removing variables not making a significant contribution to discriminant functions. Analysis of each character type showed little or no change in the mean percent correct classification of untransformed versus transformed ($\log_{10}$) data within the character types (Table 7, analyses 1 and 3). Furthermore, the removal of male posterior scutes (POS) and female anterior scutes (ANS) from meristic functions, STD and STA distances for male morphometric and the same variables for character type 3, as well as pelvic to anal distance (PTA) of males and STD distance (STD) for females produced similar, if not slightly higher, mean percent correct classification of individuals into their river of origin. Classification matrices for individual rivers are presented in Melvin (1984). Analysis of river data pooled into regions prior to LDF demonstrated virtually no difference between the raw and $\log_{10}$-transformed data when character types and grouping are compared (Table 7, analyses 4 and 5).

Given that many of the misclassified fish were assigned to a geographically adjacent river, the results (classification by stepwise LDF) of untransformed data grouped into regions after classification were examined. This meant an individual allocated to a river within a particular region was considered to be properly classified provided it was assigned to any river in the region of its origin. Examination of fish grouped in this manner demonstrated an increase in mean percentage correct classification for all three character types (Table 7, data analysis 6). Mean percent correct classification for males was found to be consistently higher than for females.

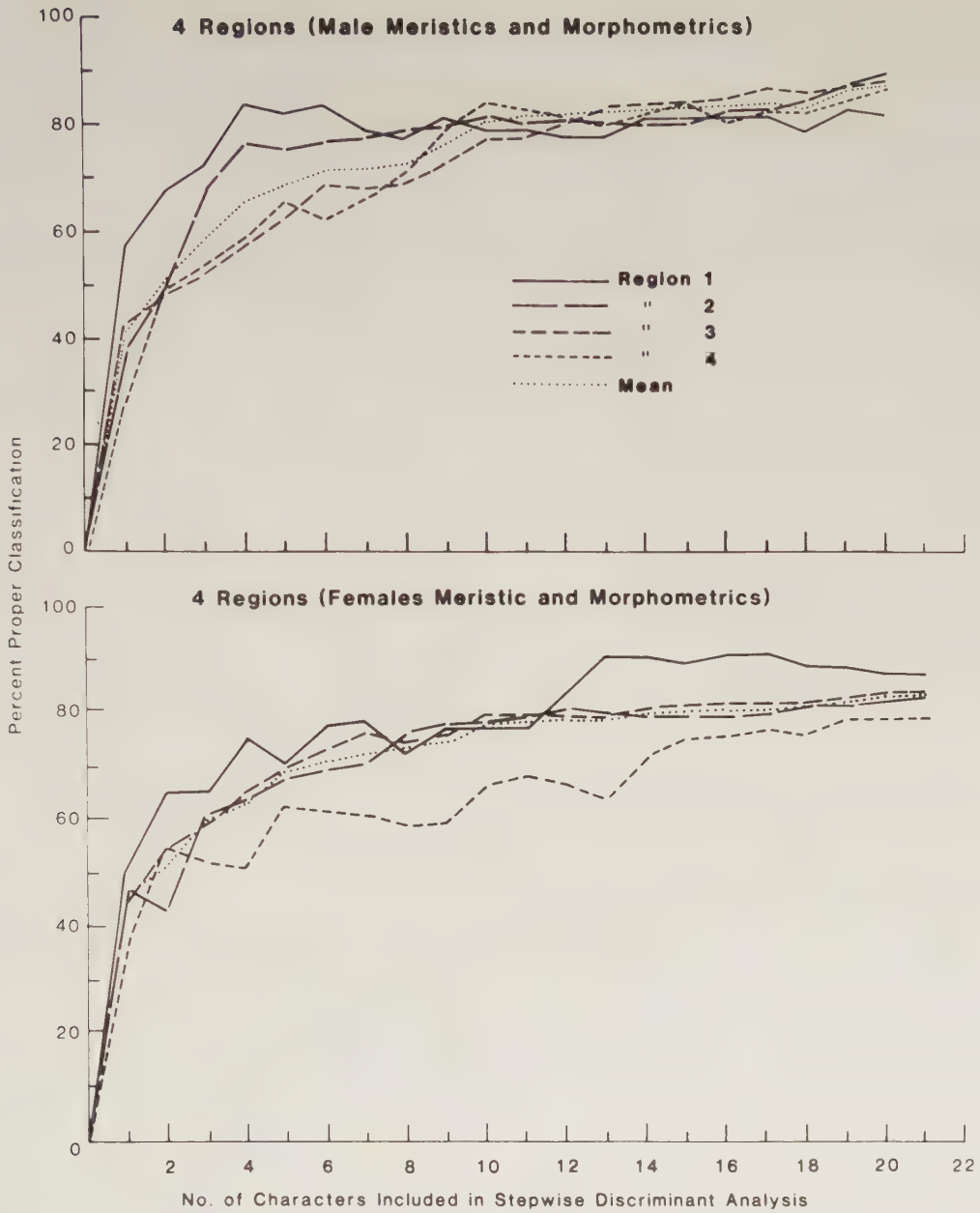

FIG. 5. Mean percent proper classification in relation to number of characters included in stepwise LDF analysis for male and female shad collected from 14 rivers and grouped into four regions.

Classification results showed a positive relationship between the number of characters used in the analysis and the mean percent properly classified (Fig. 5), with a narrowing of the distribution of misclassified fish about the river of origin. Plots of the first two canonical variates and their scatter demonstrate the degree of overlap of the four groups (Fig. 6). A clear south to north separation is, however, observed along the first canonical axis for both males and females. The two axes account for 49.1 and 14.3% of the total dispersion in males and 43.1 and 15.6% in females.

Various statistical procedures have been employed to adjust morphometric variables for differences in fish size in stock discrimination studies. These include the expression of body measurements as a ratio of length (Copeman 1977; Casselman et al. 1981) or a log-linear regression of the variable and some measurement of length (Schweigert 1981). To investigate this, we used the method adopted by Meng and Stocker (1984). Morphometric characters were adjusted for size from the linear geometric mean regression of Cumberland Basin FL versus variable as described by the above authors. Correction for length and excluding Fl from LDF analysis resulted in a less than 3% reduction of overall mean percent correct classification for both males (73.9–71.2%) and females (66.6–64.0%). The range of the 95% regional confidence intervals was estimated to be 5.8 and 6.3%, respectively, by the "leave-one-out" method (Lachenbruch 1967).

Regional assignment of river samples using character type 3 (meristic and morphometric) of untransformed and length-standardized (excluding FL) data grouped into regions, after classification by stepwise procedures into individual rivers, is shown in Table 8. The untransformed and unadjusted data were employed to develop discriminant functions for the classifica-

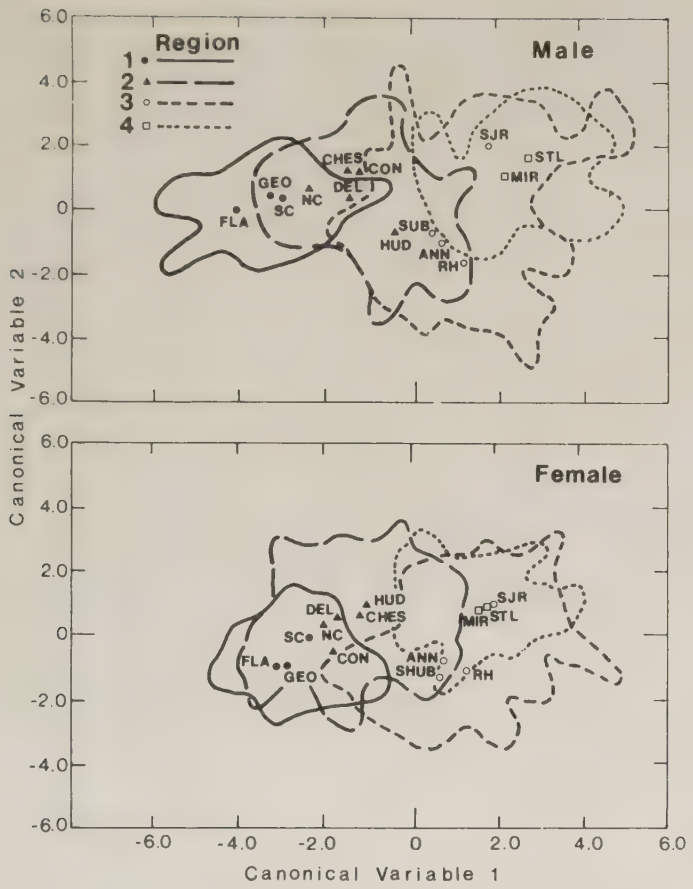

FIG. 6. Group canonical and scatter from LDF of male and female meristic and morphometric characters combined for the four regions.

tion of Cumberland Basin samples (unknown origin) into regions. Fisher's LDF coefficients for male and female shad are presented in Table 9. The characters are listed in their order of loading to the discriminant function.

Cumberland Basin Samples

The regional assignment of shad from Cumberland Basin by sex and the sexes combined is presented in Table 10. Major differences between the sexes were observed when a month's percentages were far above or below those of the opposite sex (e.g. Region 1 males in July compared with females for the same month). The subsequent pooling of male and female results was biased toward the females due to their prevalence in the samples (197 versus 461, respectively). However, a dominance of females was also noted in samples from other basins of the Bay of Fundy (M. J. Dadswell, unpubl. data) and the proportional relationship believed consistent with tagged fish. The combined data were therefore assumed to represent estimates of regional origin of both retained and marked shad collected from Cumberland Basin during the 3-yr study period: 10.9% from Region 1, 38.0% from Region 2, 32.5% from Region 3, and 18.5% from Region 4 (Table 10, male and female). The observed percentages differ significantly ($p < 0.01$) from the expected 25% assuming random assignment ($\chi^2 = 18.54$).

Standardization of LDF findings from Cumberland for comparison with tag returns required transformation of both data sets. The LDF data were represented as a percentage of shad, identified by LDF as belonging to a specific region, that were collected in each month. Presentation of the data in this form (Table 11) showed a transition of fish from June through September for Region 1. A progressive rise in the proportion from the start of the fishing to midsummer was also noted for Region 2. For the Bay of Fundy (Region 3) the maximum percent occurred in June decreasing through July and August and increasing again in September. Region 4 (Gulf of St. Lawrence) showed peak values in June and August.

Maturity

Shad collected from Cumberland Basin were identified as either adults (mature) or juveniles (immature or maturing) from visual examination of their gonads (Table 12). Of the fish sampled from Cumberland Basin and assigned by LDF to Region 1 in June, 100% were juveniles compared with 79.3% for Region 2, 59.2% for Region 3, and 51.4% for Region 4. Higher percentage values were observed for Regions 3 and 4 in June and July than for Regions 1 and 2. Maximum values of adult fish from Regions 1 and 2 never achieved levels of Regions 3 and 4, probably because of the lower incidence of repeat spawners. The general increase in percentage of adult fish throughout July and August was believed to represent the influx of mature shad whose arrival was delayed due to spawning.

TABLE 8. Male and female mean percent correct classification matrices of river samples grouped into regions after classification into rivers by stepwise discriminant analysis using meristic and morphometric variables combined. Values in parentheses are mean percent correct classification using length-standardized morphometric data (Meng and Stocker 1984) with meristic variables excluding fork length as an input parameter for LDF.

	Male					Female				
Region	No. of fish	1	2	3	4	No. of fish	1	2	3	4
1	58	81.0	19.0	0.0	0.0	44	86.4	13.6	0.0	0.0
		(82.7)	(17.2)	(0.0)	(0.0)		(88.6)	(11.4)	(0.0)	0.0)
2	129	7.8	89.2	3.1	0.0	153	7.8	82.4	7.2	2.6
		(8.5)	(86.8)	(3.9)	(0.8)		(7.8)	(81.1)	(7.8)	(3.3)
3	224	0.0	7.6	88.1	4.4	250	0.8	10.8	82.4	6.0
		(0.0)	(8.9)	(76.9)	(14.2)		(1.2)	(8.8)	(81.4)	(8.8)
4	72	0.0	2.8	11.1	86.1	53	0.0	5.6	15.1	79.4
		(0.0)	(2.8)	(18.0)	(79.2)		(0.0)	(7.5)	(17.0)	(75.5)

TABLE 9. Classification function coefficients for males and females.

Variable	SJ River	Geo River	SC River	Neu River	Sus River	Del River	Hud River	Con River	Ann River	Shub River	RHib River	SJR River	Mir River	StL River
Males														
GR	4.9875	5.2639	5.2078	5.5357	5.6564	5.4528	5.5125	5.2168	5.5095	5.2867	5.2171	6.1975	5.7849	5.7314
ML	4.5641	1.5996	−8.8135	−5.7069	−0.24167	−2.0164	−2.5870	−1.1227	8.3179	15.2992	7.8766	1.3795	4.2300	0.1356
BT	−27.4747	−26.3298	−26.4489	−23.9674	−29.5880	−26.5495	−26.3981	−29.528	−28.0220	−28.9752	−29.7417	−33.9537	−28.4145	−34.0005
TOT	12.6616	13.4150	13.2963	13.3746	14.2725	14.4675	15.8493	14.8025	14.9730	15.4922	16.0189	15.2229	15.8374	15.4825
AFB	−24.4022	−23.3561	−24.0576	−24.8024	−24.3111	−25.6144	−27.8642	−25.0365	−27.1492	−25.0199	−28.9357	−23.3937	−24.6783	−21.2493
CP	68.6852	73.3077	62.1536	62.9549	64.4950	64.4993	63.2811	71.7688	58.9517	63.0057	58.5540	64.1870	60.6281	58.5082
DVS	34.5964	33.8526	38.0215	37.6744	35.8552	35.9184	37.0167	37.4231	36.5513	37.4540	37.7748	37.6723	38.3794	37.5071
DFB	7.9413	−6.7378	−5.0419	−6.1111	−9.8421	−5.5138	−4.0946	−5.0514	−6.9470	−9.1870	−5.7604	−9.5341	−10.6228	−7.7051
FL	−0.4397	0.1171	0.4151	0.3507	0.4009	0.2292	0.0534	0.6832	0.7321	0.7369	0.2877	0.9436	2.1041	2.6585
BD	−6.0567	−5.2916	−5.1117	−4.9869	−4.4981	−6.4331	−5.0289	−8.6044	−5.5979	−5.7830	−6.0279	−6.6753	−7.7272	−7.4409
IO	24.4302	22.5998	17.8764	19.5114	24.2795	19.6219	26.7529	23.7339	17.2174	16.3827	14.1960	24.1873	21.6694	20.3537
PTP	−20.7413	−20.9157	−21.0424	−21.4614	−20.7948	−19.2036	−19.8543	−20.4048	−19.8249	−20.8631	−19.9205	−20.5964	−20.8730	−23.2361
HL	18.2127	16.0158	20.7167	19.8269	17.6347	20.7571	20.4737	18.9340	20.6466	17.7803	24.1022	21.3300	19.3588	22.4267
VERT	83.3456	81.9206	82.1118	82.4193	82.9162	83.0259	82.7006	83.8823	83.3740	83.4240	83.4896	84.5058	84.2905	84.0086
PFL	41.4953	37.6297	41.6801	41.9597	43.3093	38.5266	35.6842	38.5766	36.6691	39.0349	39.3263	36.3330	36.2288	37.1592
PFR	24.6878	24.0831	24.4731	24.1849	24.6105	24.2797	25.0930	24.7744	24.1628	24.6293	24.6252	25.0116	24.1705	24.3167
PFR	21.1663	20.6683	20.0655	19.8156	19.8450	19.4914	18.8128	18.9928	19.6247	19.8722	18.7114	20.0790	18.9699	19.5081
SL	−38.7508	32.7216	−32.8661	−39.8019	−38.9352	−35.0695	−28.8118	−29.5038	−32.8827	−34.1631	−26.4820	−33.7968	−33.9918	−32.1150
AFR	14.0956	13.9180	13.8388	14.4233	13.9800	14.3361	14.3186	14.2785	15.1231	14.8961	14.6841	14.6607	14.5538	14.3612
ANS	12.7691	13.4074	12.1953	13.6619	12.6506	12.4478	13.3383	12.9354	12.3875	11.9601	11.4333	12.2756	12.2902	12.0978
(CONSTANT)	−3934.41	−3898.77	−3934.98	−4004.96	−4026.28	−4015.32	−4109.62	−4099.14	−4101.16	−4130.06	−4124.39	−4262.06	−4215.34	−4200.73
Females														
GR	3.3481	3.5031	3.7535	3.7224	3.8473	3.8185	3.8485	3.5382	3.8644	3.7107	3.6892	4.3315	4.0216	4.0591
ML	31.5444	30.9570	25.6535	28.4697	25.7117	28.0398	30.7551	35.3508	38.4847	42.9533	36.1431	33.3708	33.1048	29.3539
BT	3.5759	1.7331	5.7464	5.0499	−0.0952	4.9716	3.6473	1.8510	3.3031	1.4934	0.3410	0.3817	2.5254	−1.6297
TOT	23.8611	22.3894	23.2949	22.3495	23.5715	23.2719	24.7106	24.0521	23.9227	23.7556	23.6940	23.9129	24.2071	24.6878
CP	81.9052	89.0710	78.6319	76.9868	79.8983	80.4542	79.8196	83.9842	76.1956	77.9021	71.4532	77.6903	75.5548	72.3544
FL	0.2557	0.1231	1.1856	1.6215	1.5362	1.0490	0.4344	0.7604	1.0510	0.4905	0.7144	0.9879	2.1974	2.9898
DVS	51.2564	50.7280	52.7721	54.0640	53.3497	52.7441	53.3895	53.1876	52.9776	53.7507	54.8275	53.9915	53.9933	52.6237
DFR	21.8663	21.8456	22.6387	22.3651	22.6526	21.1692	20.4418	21.6370	22.3363	22.1242	21.8179	22.5295	21.7103	22.9984
PTA	1.4936	1.7257	1.4172	2.3877	1.6567	2.5065	2.6437	1.7636	1.0756	1.7329	1.9485	0.3762	0.8450	0.9810
DFB	−12.1018	−11.4952	−9.5166	−9.0572	−12.2171	−9.6712	−9.4878	−10.0154	−12.5331	−12.6210	−12.2224	−13.6464	−13.9387	−14.4531
VERT	87.8316	87.4186	86.5915	88.3211	88.2931	88.5460	88.6572	88.7329	88.6560	88.5567	88.2511	89.3223	89.5364	87.6173
AFB	−18.1920	−17.5820	−17.7154	−19.0074	−19.1369	−19.9518	−19.2377	−19.7297	−20.5453	−19.8705	−22.7498	−18.5219	−19.7899	−15.1915
SL	−103.7745	−106.7741	−101.7017	−105.5160	−104.7639	−100.3603	−98.1951	−94.5540	−100.6008	−96.1847	−87.6689	−98.6854	−96.3728	−102.4965
PTP	−11.5024	−10.6192	−10.7639	−10.9565	−11.4491	10.1111	−10.5977	−10.3374	−10.8139	−10.7465	−10.4158	−11.2435	−11.8384	−12.0234
AFR	21.1597	21.3735	21.4486	21.1267	20.8723	21.5920	21.3380	21.6034	22.2878	22.2748	22.0804	21.8490	21.4369	21.1961
IO	−2.5333	−3.9382	−4.5003	−4.2243	−3.4346	−3.5854	−3.2640	0.2086	4.4588	−4.8858	−3.8660	−3.3765	−4.1404	−4.5479
HL	27.4845	29.3920	29.1125	26.2559	29.0008	27.3558	29.2625	24.4733	29.7352	27.9774	30.7381	29.0267	28.0928	30.8208
POS	−16.6262	−13.8613	−16.5090	−15.1820	−15.8203	−15.0623	−15.5001	−16.0682	−15.6582	−15.2283	−14.2654	−14.7542	−14.7492	−15.5516
PFL	39.0968	39.2914	38.5340	42.1115	39.7251	37.8140	37.4039	36.3903	39.1443	39.4315	39.4075	39.9295	37.6806	38.5467
PFR	14.3879	14.3418	14.6212	14.6756	14.1276	14.0930	14.6000	14.2746	14.2981	14.4221	14.3781	14.7697	14.2151	14.6202
STA	−7.6943	−8.0600	−9.0208	−9.4355	8.3333	−8.7995	−9.0316	−8.4700	−8.8483	−8.8430	−8.7565	−8.4811	−8.9024	−9.2266
(CONSTANT)	−4052.66	−4051.19	−4077.25	−4166.40	−4157.93	−4173.97	−4230.22	−4189.87	−4253.16	−4242.91	−4240.55	−4356.51	−4319.10	−4247.03

TABLE 10. Percentage of fish sampled in each month and classified into each region, using meristic and morphometric characters, after classification to individual rivers by discriminant functions. The percentage from region column represents the overall percentage of fish sampled from the Cumberland Basin. The values in parentheses are the number of fish.

Sex	Region	No. of fish	Month				% from region
			June	July	August	September	
Male	1	15	7.4 (4)	3.9 (3)	10.9 (5)	14.3 (3)	7.6
	2	83	27.8 (15)	48.7 (37)	52.2 (24)	33.3 (7)	42.1
	3	58	48.1 (26)	23.7 (18)	17.4 (8)	28.6 (6)	29.4
	4	41	16.7 (9)	23.7 (18)	19.5 (9)	23.8 (5)	20.8
Female	1	57	8.3 (10)	13.9 (27)	12.8 (12)	15.4 (8)	12.4
	2	167	31.4 (38)	41.2 (80)	34.0 (32)	32.7 (17)	37.0
	3	156	37.2 (45)	31.4 (61)	29.8 (28)	42.3 (22)	34.6
	4	81	23.1 (28)	13.5 (26)	23.4 (22)	9.6 (5)	18.0
Male and female	1	72	8.0 (14)	11.1 (30)	12.1 (17)	15.1 (11)	10.9
	2	250	30.3 (53)	43.3 (117)	40.0 (56)	32.9 (24)	38.1
	3	214	40.6 (71)	29.3 (79)	25.7 (36)	38.4 (28)	32.5
	4	122	21.0 (37)	16.3 (44)	22.1 (31)	13.7 (10)	18.5

TABLE 11. Summary of male and female data combined and transformed from Table 9 to represent the monthly percentage of fish classified into each region.

Region	Month				Total %
	June	July	August	September	
1	17.3	24.0	26.1	32.6	100
2	20.5	29.6	27.3	22.5	100
3	30.3	21.9	19.1	28.6	100
4	28.7	22.3	30.2	18.8	100

Tag Returns

Of the 6124 shad marked in Cumberland Basin during the 3-yr study period, 353 or 5.8% were recovered prior to November 30, 1983. Geographically, fish were reported in coastal and river waters from the St. Johns River, Florida, to the St. Lawrence River, Quebec (Fig. 7). Returns were summarized by state/province and habitat of recapture (freshwater/marine) for each year of tagging (Table 13). However, because shad recaptured seaward of freshwater boundaries could not be considered indigenous to a specific river, they were excluded from computational procedures used to compare LDF and tag return findings.

The transformation of tag return data to represent values synonymous with LDF results required that river recaptures first be expressed as a percent of monthly releases. These values illustrate both regional and monthly percentage recapture (Table 14). Returns from U.S. rivers were in the order of 5–6 times those from Canadian waters. Several factors were assumed to be responsible for this finding: size of U.S. populations, fishing effort, and interest sparked by a Canadian return address, especially in the southern states. Maximum percent river returns were reported for fish tagged during August and not July when the greatest number of shad were marked (Table 14).

The disparity of tag returns was adjusted to account for differences by transforming each region's monthly percentage recaptures to a percentage of the total regional percentage recaptures (Table 15). The values computed represent the percentage of regional percentage of shad recaptured in a region that were tagged during a specific month of the sampling period. Hence, they depict the seasonal transition of fish moving through Cumberland Basin from each region and are equivalent to values in Table 10.

Examination of return data in this form disclosed regional specific monthly peaks. Of the fish subsequently recaptured from rivers of the northern regions (3 and 4), the greatest percentages were marked in June. As summer progressed, the percentages decreased for Regions 3 and 4 while increasing for fish recaptured south of Cape Cod until August. In September the northern region percentage again increased. Region 1 percentages continued to increase throughout the summer.

Discussion

Previous studies of the American shad have utilized meristic and, to a lesser extent, morphometric characters to investigate the relative contribution of major spawning rivers to specific fisheries (Fischler 1959; Hill 1959) or the statistical significance of these characters to identify discrete river populations (Warfel and Olsen 1947; Nichols 1966; Carscadden and Leg-

TABLE 12. Percentage of adult (mature) and juvenile (immature and maturing) shad collected from Cumberland Basin and classified into each region by discriminant functions. The values in parentheses are the number of fish.

	June		July		August		September	
Region	Juvenile	Adult	Juvenile	Adult	Juvenile	Adult	Juvenile	Adult
1	100.0	0.0	76.7	23.3	52.9	47.1	81.8	18.2
	(14)	(0)	(23)	(7)	(9)	(8)	(9)	(2)
2	79.3	20.7	59.8	40.2	57.1	42.9	54.2	45.8
	(42)	(11)	(70)	(47)	(32)	(24)	(13)	(11)
3	59.2	40.8	45.6	54.4	41.7	58.3	39.9	60.7
	(42)	(29)	(36)	(43)	(15)	(21)	(11)	(17)
4	51.4	48.6	31.8	68.2	35.5	64.5	10.0	90.0
	(19)	(18)	(14)	(30)	(11)	(20)	(1)	(9)

gett 1975). Similar approaches were adopted for the striped bass by Lund (1957) and Raney and DeSylva (1953). In general, these studies were restricted, concentrating on the differences between a few of the major rivers, the exception being Nichols (1966). Our study represents a multivariate approach to the investigation of the usefulness of meristic and morphometric characters for discriminating mixed populations of American shad from a marine environment now known to contain fish indigenous to virtually every shad spawning river along the Atlantic coast (Dadswell et al. 1983, 1987). In addition, the results are supported by an independent, but concurrent, tagging program.

The regional classification of all fish collected during the 3-yr study period by LDF analysis indicated the largest proportion (38.0%) originated from rivers between Cape Lookout and Cape Cod (Region 2) with 32.5% from rivers of the Bay of Fundy (Region 3), 18.5% from rivers of the Gulf of St. Lawrence (Region 4), and 10.9% from rivers south of Cape Lookout (Region 1). These results correspond to what might be expected from examination of the regional landing statistics, migratory patterns, and life history of this species. Region 2 contains the largest spawning populations of American shad along the Atlantic coast (Walburg and Nichols 1967) and therefore should dominate Cumberland Basin catches, assuming migratory patterns are similar for all regions. On the other hand, the concentration of Region 3 fish, which contains Cumberland Basin, may well be a function of the presence of pre- and post-spawning shad. For Region 4, the distance from rivers around Nova Scotia to the inner Bay of Fundy is comparable with the distance from Region 1. In addition, oceanographic conditions similar to the inner Bay of Fundy (turbid and highly productive) exist at the mouth of the St. Lawrence River (D'Anglejan 1981); hence, many of the shad from this region may utilize the nearby area instead of undertaking extensive southern migrations.

The life history of shad from Region 1 had been described as distinctly different (Glebe and Leggett 1981) from the other regions and may help to explain their low percentage and the presence of predominantly juvenile fish in Cumberland Basin catches. The absence of repeat spawners (<5%) suggests most of the adults die after spawning. This fact alone would reduce the percent of adult fish present at sea and supports the finding of 76.0% juveniles from Region 1 in Cumberland Basin.

The transformation of Cumberland Basin LDF analysis and tag return data into regional monthly percentages provided two independent mechanisms for the determination of shad origin. The results of these two approaches were similar in that both demonstrated the same general trends throughout the fishing season. In essence, tag return data furnished direct evidence that shad occurring in the Basin migrate to the various rivers for spawning, and the similarity of monthly percentages with LDF analysis findings supports the regional assignment of individuals by the discriminant functions. Noted differences between the two approaches were probably the result of the lower number of tag returns from Regions 3 and 4 for August and September and the fact that tagging operated on a continuous basis whereas the collection of specimens for meristic and morphometric analysis were point samples with respect to time. The percent of tag returns was, however, approximately proportional to the 1982 reported landings for each region (R. Smith, Department of Fisheries and Oceans, Statistical Division, pers. comm.; J. Boreman, National Marine Fisheries Service, pers. comm.).

The findings illustrate a seasonal transition in the proportion of fish from given areas as summer progressed. In general, of the shad recaptured or classified by LDF into waters south of the Bay of Fundy, a greater proportion were marked or captured during midsummer (July–August) in contrast with June and September for the Bay of Fundy and north.

The results of this study suggest, at least for adults, that the summer progression of shad through Cumberland Basin may be related to their place of origin and the distance travelled. Shad from Bay of Fundy rivers have a shorter distance to travel upon completion of spawning, and therefore should arrive in Cumberland Basin earlier than fish from more southern or northern rivers. However, distance from river of origin cannot account for the increased presence of Gulf of St. Lawrence shad in June or the smaller proportion from Regions 1 and 2. Maturity data indicated that shad collected from Cumberland Basin and identified by LDF to originate from Region 1 were immature and that there was a concurrent increase in the ratio of immature to mature shad southward. Also for Region 4, a large proportion of adult shad were present at a time when they should have been spawning. Apparently, these shad would not have spawned in the year of capture. Other observed anomalies included the reduced overall catches in August and the decrease in Region 2 and increase in Region 3 shad during September. It is probable that other factors such as water temperature, turbidity, and different migration routes also play a role in the timing and duration of stay of shad in Cumberland Basin (Dadswell et al. 1983, 1987). Both inherited and/or ontogenetically acquired temperature preferences contribute to isolate stocks and species in space and time (Reynolds 1977). Consequently, different temperature preferences could be expected for shad, originating over a wide latitudinal range. If so, as temperature

FIG. 7. Recapture totals for tag returns from rivers from 6124 shad marked in Cumberland Basin between 1979 and 1981.

warms beyond the preferred range for shad from the northern regions (3 and 4), their proportions decline, increasing again when temperature declines.

Various levels of success have been achieved using meristic and morphometric characters to discriminate populations of fishes. Sharp et al. (1978) examined nine meristic and 11 morphometric characters to classify capelin (*Mallotus villosus*) from the Northwest Atlantic into five groups. Using four variables, their a posteriori classification was 93.1% for males and 73.3% for females. On the Pacific coast, Meng and Stocker (1984) were able to correctly classify 55.3% of Pacific herring (*Clupea harengus pallasi*) into six groups and 85–90% into two groups using 17 characters. In comparison, Schweigert (1981) was able to classify only 67.0% into three groups using 11 meristic and morphometric characters. Our results compare relatively well with these studies but they cover a much wider geographic area.

To summarize, we conclude that meristic and morphometric characters provide a useful and reliable means to discriminate individuals and/or groups of Atlantic shad inhabiting Cumberland Basin. Furthermore, they provide a method for estimating the potential impact of proposed tidal power development on Atlantic coast shad populations.

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TABLE 13. State/province summary of Cumberland Basin tag returns by river and marine (parentheses) environments for each year of sampling.

State/Province	Year		
	1979	1980	1981
Florida	2 (1)	6 (4)	8 (3)
Georgia	1 (0)	11 (1)	18 (0)
South Carolina	1 (0)	11 (1)	12 (6)
North Carolina	3 (0)	8 (4)	6 (4)
Virginia	2 (0)	7 (7)	4 (5)
Maryland	9 (0)	1 (1)	0 (1)
Delaware	1 (0)	7 (3)	1 (2)
Pennsylvania	0 (0)	3 (0)	13 (0)
New Jersey	1 (0)	4 (5)	11 (8)
New York	0 (0)	4 (2)	10 (2)
Connecticut	2 (0)	3 (0)	6 (0)
Massachusetts	2 (0)	0 (2)	1 (1)
New Hampshire	0 (0)	0 (0)	0 (1)
Maine	0 (0)	0 (2)	9 (0)
Nova Scotia	0 (4)	6 (4)	6 (2)
New Brunswick	0 (17)	5 (36)	1 (23)
Quebec	2 (0)	4 (1)	2 (1)
Totals	18 (22)	80 (76)	99 (59)
Total tagged	494	2361	3269
% recapture	3.6 (4.5)	3.4 (3.2)	3.0 (1.8)
Total % recaptured		353/6124 = 5.8	

TABLE 14. Cumberland Basin tag returns expressed as a percentage of the total number of fish tagged monthly during the study period. The actual number of returns is enclosed in parentheses.

Region	Month				Total	Regional %
	June	July	August	September		
1	0.67 (8)	1.35 (50)	1.52 (12)	1.60 (7)	5.14	1.29
2	0.92 (11)	1.56 (58)	2.28 (18)	1.39 (6)	6.15	1.54
3	0.42 (5)	0.22 (8)	0.13 (1)	0.46 (2)	1.23	0.31
4	0.25 (3)	0.16 (6)	0.13 (1)	0.23 (1)	0.77	0.19

TABLE 15. Percentage of regional percentage of tag returns by month.

Region	Month			
	June	July	August	September
1	13	26	30	31
2	15	25	37	23
3	34	18	11	37
4	33	21	17	29

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Genetic Models for Cyclic Dominance in Sockeye Salmon (*Oncorhynchus nerka*)

Carl Walters

Fisheries Centre, University of British Columbia, Vancouver, B.C. V6T 1W5, Canada

and J. C. Woodey

Pacific Salmon Commission, 1155 Robson St., Vancouver, B.C. V6E 1B9, Canada.

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A simple genetic mechanism may be partly responsible for maintaining violent cycles in abundance of some Fraser River sockeye salmon (*Oncorhynchus nerka*) stocks. If age at maturity is highly heritable, spawning runs in low cycle lines may have high percentages of age 5 spawners that tend to produce offspring that will return at age 5, due to production of low percentages of such spawners by preceding high cycle lines. Then even if each low cycle line has a high productivity per spawner, a large fraction of this productivity may be "lost" to the cycle line, in the form of age 5 recruits to other cycle lines. In the face of high fishing mortality, even a small relative loss from any cycle line may cause it to remain small relative to other lines. This model explains several observations, such as high productivity per spawner in low cycle lines, that cannot be explained by previous hypotheses involving depensatory predation or food supply. However, it is unlikely that genetic effects alone are responsible for the stability of cyclic patterns, unless low offcycle runs consist entirely of "temporal colonizer" genotypes that produce a high proportion of age 5 offspring as a dominant trait.

Un simple mécanisme génétique peut être en partie responsable des cycles marqués de l'abondance chez certains stocks de saumon rouge (*Oncorhynchus nerka*) du fleuve Fraser. Si l'âge à la maturité est une caractéristique fortement héréditaire, les remontées de lignées à faible cycle peuvent contenir un pourcentage élevé de géniteurs de 5 ans qui ont tendance à produire une progéniture qui reviendra frayer à l'âge de 5 ans, à cause de la production de faibles pourcentages de tels géniteurs par les lignées précédentes à cycle élevé. Par conséquent, même si chaque lignée à faible cycle montre un taux élevé de productivité par géniteur, un pourcentage élevé de cette productivité peut être "perdue" pour ce cycle sous la forme de recrues de 5 ans recrutées à d'autres cycles. Dans le cadre d'un taux élevé de mortalité par pêche, même une faible perte relative dans toute lignée peut signifier qu'elle restera peu abondante par rapport à d'autres lignées. Le modèle élaboré dans la présente étude explique plusieurs observations, comme le taux de productivité élevé par géniteur de lignées à faible cycle, qui ne peuvent être expliquées par des hypothèses précédentes portant sur la prédation anticompensatoire ou l'apport en nourriture. Toutefois, il est peu probable que des facteurs génétiques soient les seuls responsables de la stabilité des régimes cycliques à moins que les remontées hors cycle soient uniquement composées de génotypes du genre "colonisateurs temporels" qui produisent un pourcentage élevé de saumons de 5 ans comme trait dominant.

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One of the most striking patterns in the population biology of Pacific salmons is the violent cyclic behavior of some sockeye (*Oncorhynchus nerka*) populations, especially in the Fraser River. A major challenge for salmon biologists is to explain these cycles, and the explanation may have great economic significance if it turns out to involve a mechanism that can be manipulated through management to produce larger runs in all years.

The violent cycles have a period equal to the dominant age at return, which is 4 yr in the Fraser. Each of the four spawning years (or relatively distinct temporal subpopulations in a single stream) is referred to as a "cycle line." In a typical 4-yr cycle line, the fish spend 2 yr in freshwater rearing lakes and then two summers at sea before returning in the fourth calendar year after they were spawned. Large cycle line spawning runs tend to produce large recruitments, while low lines produce low recruitments. While some researchers have argued that such cycles can be explained most simply as resulting from depensatory overfishing on the low lines (Eggers and Rogers 1987; Walters and Staley 1987; Collie and Walters 1987), others have argued that the cycles were present historically and must be maintained by some mechanism of "cyclic dominance" that tends to prevent low cycle lines from increasing. Ward and Larkin (1964) and Larkin (1971) suggested that the mechanism might be depensatory predation, with predation rates related to abundance of juveniles in the dominant cycle lines such that survival improves when the dominant line is depressed. But data gathered by the International Pacific Salmon Commission indicate that low cycle lines often have higher smolt and adult recruitment rates per spawner than do high cycle lines (Cass 1989; Vernon 1982). Another suggestion is that high smolt abundances from strong lines may affect the abundance or composition of plankton food supplies available to juveniles in subsequent years; again, field sampling has not supported this hypothesis (Ward and Larkin 1964; I. Williams, pers. comm.).

TABLE 1. Patterns of escapement and female spawning age composition for major Fraser River sockeye stocks. The stocks below comprise roughly 90% of the total spawn production. Percentages of age 4 females for each cycle line from 1960 to 1987 are shown in parentheses; remaining females are mainly age 5. Spawning numbers are thousands of age 4 and older adults, averaged over 1948–85 (from Cass 1989).

Stock	Cycle year			
	1984	1985	1986	1987
Nadina	1.7	18.2	2.0	14.3
	(67.6)	(98.4)	(90.5)	(99.7)
Gates	14.3	1.8	0.2	2.5
	(99.6)	(92.6)	(65.1)	(99.3)
Seymour	5.8	7.1	42.6	34.0
	(87.0)	(92.7)	(98.5)	(97.6)
Scotch	0.1	5.3	1.1	0.2
	(100)	(100)	(99.4)	(53.9)
Horsefly	0.3	380.8	6.0	0.3
	(75.2)	(100)	(75.6)	(63.8)
Chilko	472.7	75.3	120.8	308.7
	(96.4)	(64.0)	(97.0)	(93.9)
Stellako	51.5	43.8	93.4	120.5
	(89.0)	(85.3)	(94.7)	(97.2)
Birkenhead	57.3	35.4	56.6	38.0
	(75.7)	(66.3)	(76.3)	(61.3)
Adams	4.3	3.2	1743.1	252.2
	(80.4)	(91.6)	(99.8)	(97.9)
Lower Shuswap	0.0	1.7	101.1	5.4
	(64.0)	(100)	(99.8)	(81.7)
Weaver	27.7	30.2	63.8	22.1
	(96.2)	(94.0)	(88.2)	(77.5)
Portage	0.8	2.3	12.2	2.4
	(94.9)	(99.6)	(99.6)	(92.8)
Cultus	11.6	6.4	17.1	23.6
	(90.1)	(95.4)	(100)	(98.9)
Late Stuart	2.0	272.5	13.5	8.3
	(82.0)	(99.9)	(74.1)	(91.7)

The key observation that remains unexplained is why some cycle lines are relatively stable at very low spawning numbers, in spite of apparently good smolt quality and high recruitment rates per spawner (Walters and Staley 1987). There are three possible ways to explain this observation: (1) recruitment rates for low cycle lines are not really high, and only appear to be so because of errors in recruitment assessments (Walters and Staley 1987); (2) recruitment rates per spawner are high, but are associated with differentially high fishing mortality rates (depensatory fishing hypothesis); or (3) recruitment rates per spawner are high, but a significant proportion of the recruits are lost to other cycle lines (e.g. recruit at age 5).

It is important to recognize from the outset that violent and persistent cyclic behavior is not in fact characteristic of most sockeye salmon stocks, even in the Fraser River watershed. The roughly 20 stock complexes (collections of spawning runs using a common rearing lake) in the Fraser exhibit at least four distinct population patterns (Table 1). No persistent cycles are seen at all in the Birkenhead, Chilko, Harrison, Pitt, and Weaver Creek stocks; these stocks tend to have high percentages of age 5 spawners, which by itself would break up any cyclic pattern that might be initiated by some event such as a natural disaster. Cycles have just appeared recently during population declines in the Bowron, Cultus, and Raft River stocks. There have been cyclic patterns but with changes in dominant lines in the Early Stuart, Nadina, and Stellako stocks. There have been strong cyclic patterns with a dominant line followed by a subdominant line and then two low "offcycle" lines (DSLL pattern) in the Fennell Creek, Gates Creek, Horsefly, Mitchell, Portage Creek, and Scotch Creek stocks, but in all these cases there has recently been a "spreading" of fish to the smaller cycle lines so that these lines are increasing. Stable cycles with the DSLL pattern are seen only in the Lower Adams, Lower River Shuswap, Late Stuart, and Seymour River stocks. These stocks, along with the stocks that are now showing spreading of fish to the offcycles, are the focus of speculation about mechanisms of cyclic dominance and will be our primary concern in the following discussion.

Here we hypothesize that violent cycles of the DSLL type are due to a combination of high fishing mortality rates and a very simple genetic mechanism involving inheritance (or maternal effects as suggested by Bradford and Peterman 1987) of age at maturity. While the classic view of Fraser River sockeye is that they mature mainly at age 4, many stocks have significant proportions of age 5 spawners. These age 5 spawners represent a mixture of fish that have spent two or three summers at sea, with the three-summer pattern predominating. In particular, DSLL stocks show a cyclic pattern in the proportion of age 5 spawners, with the proportions increasing from lowest in the dominant year to highest in the first low year. Essentially, what we suggest is happening is that larger cycle lines are "dumping" a small percentage of their recruits as age 5 type spawners into the smaller cycle lines. These small percentage losses from the large lines then make up a larger percentage of the spawners in the smaller lines, which then causes the smaller lines to "lose" productivity (in the form of age 5 recruits) to subsequent cycle lines. In an extreme version of this hypothesis, the lowest cycle lines may consist entirely of genetic 5-type "temporal colonists" that have invaded the low cycle lines following extinction events.

While a genetic model for cyclic dominance is simple and intuitively appealing, there are two key difficulties with it. First, it may require unrealistic patterns of inheritance for age at return. If heritability is low, the age 5 spawners added to low cycle lines should produce high percentages of age 4 progeny, thus helping the low cycle lines to increase rather than helping to keep them low. Second, the genetic model may fail to explain long-term persistence of cyclic behavior; it may produce only a short-term "depensatory" effect, such that accidental increases in the abundance of 4-type fish in a low cycle line will reduce the swamping effect of 5-types and release the 4-type fish to increase more rapidly.

In the following sections we first review evidence concerning the apparent heritability of age at return and then use graphical and simulation models to examine the stability of cyclic patterns under alternative assumptions about the inheritance mechanism. We conclude with a discussion of the implications of the genetic model for management efforts aimed at rebuilding offcycle runs.

Apparent Heritability in Age at Return

General correlations between parent and progeny age at maturity have been documented for Bristol Bay sockeye (Rogers 1987), and Bradford and Peterman (1987) have shown that these patterns can be explained as a maternal rather than genetic effect. Similar patterns are apparent in some Fraser River sockeye stocks. Heritable differences in growth rate have also been found (Wood and Foote 1990), and these differences

could affect patterns of maturation and age at return as well (but see Healey 1987).

Figure 1 shows proportions of age 5 spawners and resultant progeny over time for the larger Fraser River stocks, along with patterns of adult (2 and 3 ocean summer) spawner abundance. The data indicate that high proportions of age 5 spawners are found mainly in offcycle lines for those stocks that show clear cycles; however, the data do not idicate that the proportion of age 5 progeny closely tracks the parental age 5 proportion for the cyclic stocks. The parental proportions shown in Fig. 1 were estimated by otolith sampling of spawners, usually involving collection for each run shown of at least 120 otoliths per year for each sex. However, sample sizes were often much smaller for small offcycle runs, and this may explain the general lack of parent–offspring correlation in stocks such as the Adams and Late Stuart. Proportions of age 5 offspring were estimated from the otolith samples and from scale samples taken from the catch for racial identification. Again, the age 5 offspring proportions from scale sampling are likely to be very inaccurate, particularly for the second offcycle line in stocks such as the Adams; for second offcycle spawnings, age 5 recruits are sampled as very small percentages of the dominant runs with which the age 5 fish are returning. Possible effects of ageing errors when sampling the dominant runs are illustrated by the Lower Shuswap data in Fig. 1, where it appears that progeny from the second offcycle line have consisted almost entirely of age 5 fish; these fish were mainly individuals aged as 5_3 (2 ocean, 3 freshwater), and actually might have been age 4_2 fish with a false freshwater annulus.

The data in Fig. 1 suggest that production of age 5 offspring is controlled at least partly by some environmental factor that is shared among stocks. Several stocks have shown especially high proportions of age 5 spawners since the 1980 brood year, mainly in offcycle spawning runs. For one major stock (Adams), high proportions have coincided with a decrease in marine growth rate as indicated by average length of returning females (Fig. 2).

Cyclic variation in sizes of adult 4_2 females would indicate density dependence in ocean growth, which might in turn influence the proportion of fish maturing at age 4; however, no such cyclic variation has been observed (Fig. 2). Likewise, smolt size might influence age at maturity. While smolt sizes have not been consistently sampled throughout the watershed, there are good time series of freshwater circulus counts from scales collected for stock identification, and Clutter and Whitesel (1956) have shown that smolt size and circulus count are closely correlated. The circulus count data indicate clear cyclic variation in smolt sizes only for the Adams and other stocks that rear in Shuswap Lake, although there is a general tendency for larger spawning runs to result in smaller smolts throughout the watershed (Fig. 2). Interestingly, larger smolt sizes in Shuswap are associated with cycle lines that tend to return more at age 5; if growth rate and age at maturity were inversely correlated as a phenotypic response, we would expect to see the opposite pattern.

Note that the data in Fig. 1 include both DSLL cyclic and noncyclic stocks, yet the same general pattern is apparent. Higher proportions of age 5 spawners generally result in higher proportions of age 5 progeny (Fig. 3), although the parent–progeny correlation is statistically significant only for a few stocks such as the Birkenhead that do not show clear cycles in escapement. The parent–progeny relationships generally show disproportionately high production of age 5 progeny when parental proportions are low. There are three possible explanations for this pattern: (1) production of age 5 progeny may be controlled largely by environmental factors, independent of parental proportion; (2) production of age 5 progeny may be overestimated due to ageing errors (false annuli); or (3) the pattern would be expected if the mating system is random, only one or a few loci code for age at return, and if the 4-type allele combinations are recessive so that most heterozygotes return at age 5.

A key feature in the field data is the tendency for the second offcycle year to have lower proportions of age 5 spawners in DSLL stocks. This is expected under both genetic and nongenetic models that predict nearly constant proportions of age 5 offspring, due to reduced input of 5-type fish to the second offcycle from the first (which is also small but receives relatively more 5-type fish from the subdominant run which precedes it). However, the second offcycle line is often smaller in DSLL stocks than the first offcycle line; this would not be expected if the only source of offspring loss were through matings with 5-type fish from the previous cycle line. The second offcycle lines also tend to produce lower proportions of age 5 offspring than the first offcycles, so the relatively lower abundance of the second offcycles cannot be explained only by recruitment loss to other cycle lines; either the second offcycle lines are suffering differentially high fishing mortality rates as well, or else recruitment rates for them have been overestimated.

Predictions of Population Behavior

This section develops quantitative models for the interaction between population dynamics and the inheritance of tendency to return at age 5. We first develop two simple models for the inheritance pattern, based on parent–offspring correlation and on a single-locus Mendelian hypothesis. We apply these models in conjunction with the Ricker stock–recruitment equation as an empirical description of overall productivity and density-dependent effects. The Ricker model appears to give good general predictions of how average recruitment rates vary over wide ranges of spawning stock size for Fraser sockeye (Collie et al. 1990; Walters and Staley 1987; Cass 1989; Welch and Noakes 1990).

Note that in the models below we do not consider one obvious genetic interaction among cycle lines that could cause variation in proportions of offspring returning at age 5. Relatively large numbers of age 3 males (compared with the number of older females) often return on the weak year preceding the dominant cycle year. It is conceivable that there is some genetic basis for the tendency to return at age 3 and that matings with these males could produce high proportions of age 5 female offspring. However, such a mechanism cannot explain the occurrence of two offcycle years in a row, since the first of these lines is not subject to the high input of young males (such males would have to come from the previous spawning of the second offcycle line).

Models for Inheritance of Age at Return

Here we suggest two models for the relationship between the proportion of age 5 spawners s_5 and their progeny p_5. "Model A" says nothing about the genetics of offspring production and might describe a maternal or genetic effect. It is motivated by the empirical patterns reported by Rogers (1987) and Fig. 3 and

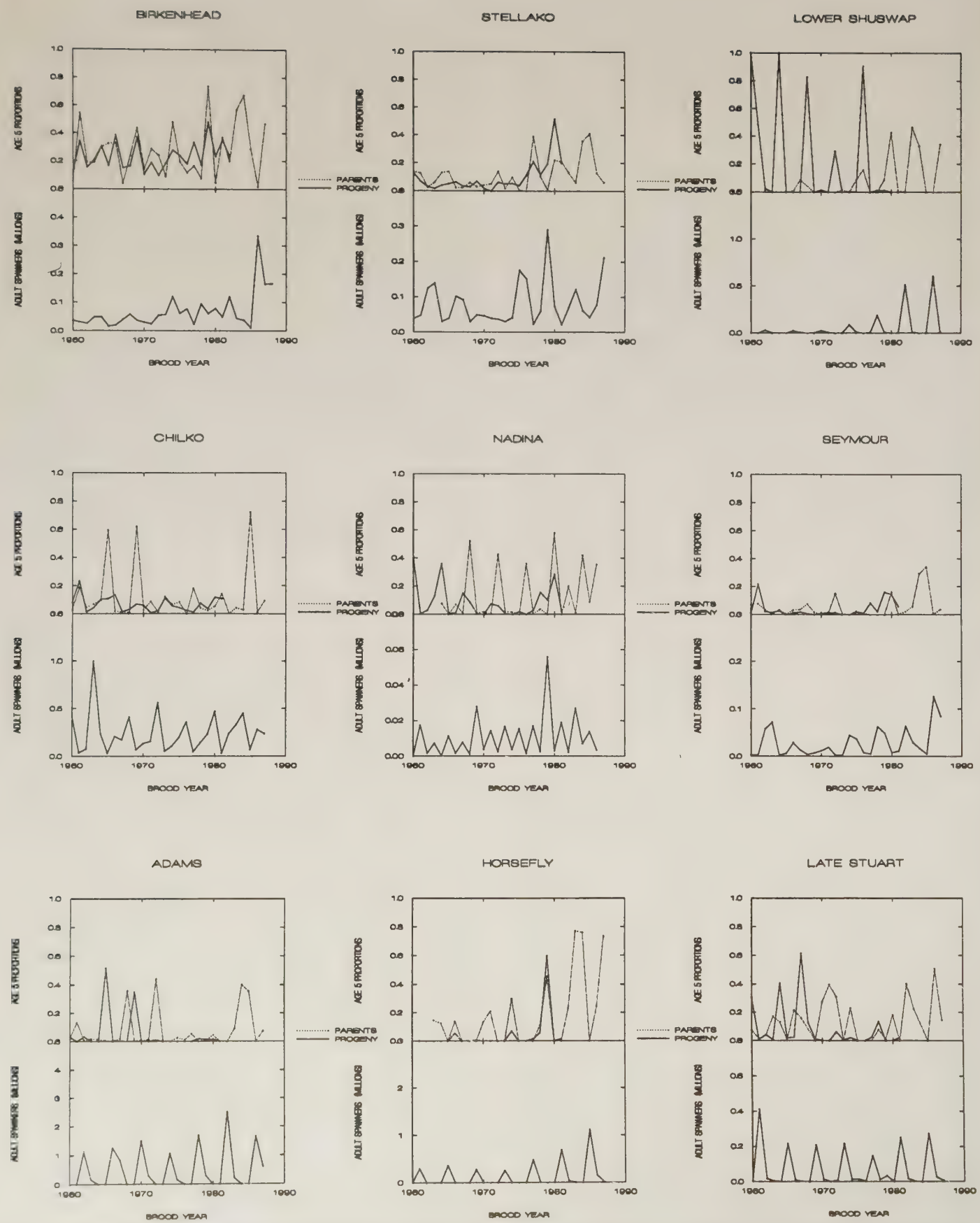

FIG. 1. A sampling of the variety of temporal behaviors exhibited by Fraser River sockeye stocks. Note that age 5 proportions are plotted by brood (spawning) year rather than year of return.

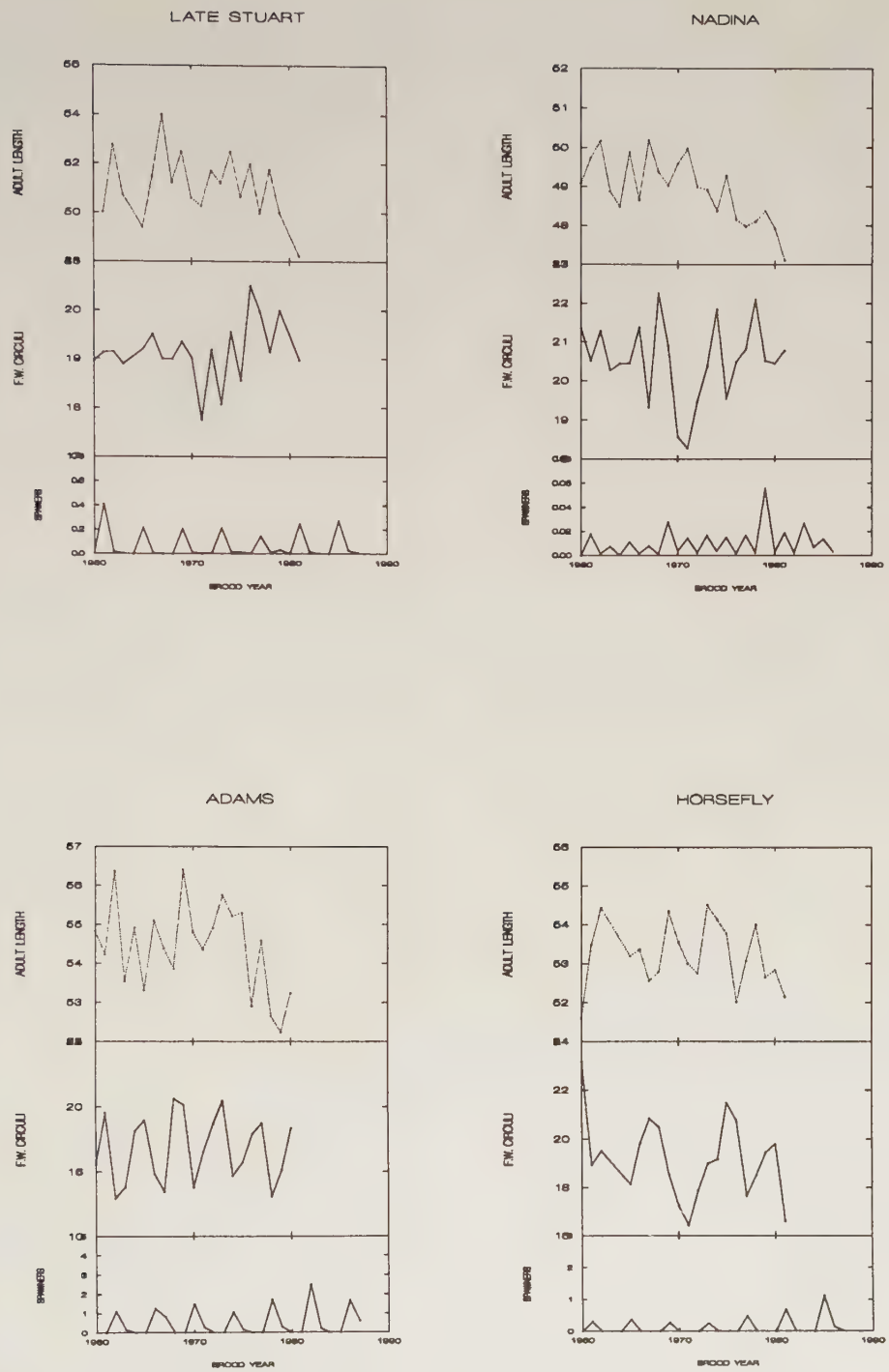

FIG. 2. Variation in standard lengths of adult age 4_2 females and in smolt sizes as indicated by freshwater circulus counts on scales sampled from returning adults.

assumes simply that parental and progeny proportions are linearly related with a mean progenal proportion $p_5 = 0.5$ when the parental spawning proportion is $s_5 = 0.5$:

(1) Model A: $p_5 = 0.5 + i\,(s_5 - 0.5)$.

Here the slope parameter i measures the strength of the parent–offspring correlation: $i = 0$ implies no parent–offspring correlation, $i = 1$ implies perfect correlation, and $i < 1$ implies some regression toward the mean (0.5) even when s_5 is very small or large.

"Model B" assumes that age at maturity is determined by a single locus with two alleles A and a. Mating is assumed to be random. Progeny of genotype AA are assumed to have a probability p_{AA} of returning at age 5, progeny of genotype Aa a probability p_{Aa} of returning at 5, and progeny of genotype aa a probability p_{aa} of returning at 5. In this scheme, $p_{AA} = 1$, $p_{Aa} = 1$, and $p_{aa} = 0$ would represent perfect dominance of the A allele in coding for return at age 5. Any parental phenotypic proportion s_5 may produce a variety of progenal phenotypic proportions p_5 depending on the proportions of genotypes represented in each parental age group. To simulate

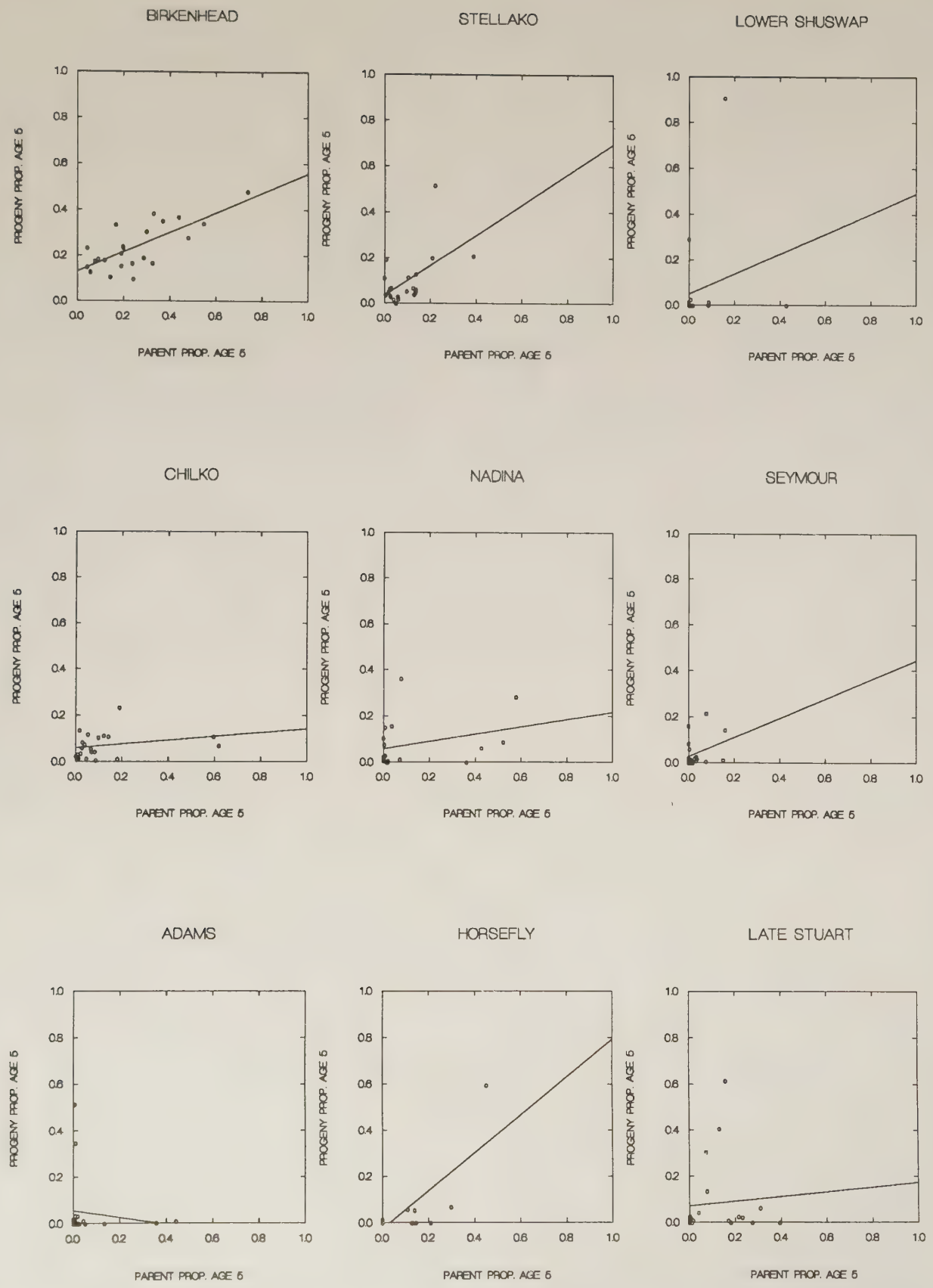

FIG. 3. Parent–progeny correlations in the proportions of age 5 fish (same data as Fig. 1). Progeny proportions were measured on returning fish, without correction to a common recruitment age.

this model, it is necessary to keep track of the genotypic proportions g_{AA}, g_{Aa}, and g_{aa} for parents of each age in the spawning runs over time. Letting $s' = g_{AA} + 0.5g_{Aa}$ be the genotypic proportion of A alleles in the spawners (of both ages combined), the phenotypic proportion of age 5 progeny under random mating will be given by

(2) Model B: $p_5 = s'^2 p_{AA} + 2s'(1 - s')p_{Aa} + (1 - s')^2 p_{aa}$.

Here the products involving s' are simply the Hardy–Weinburg proportions of offspring by genotype given parental gene frequency s', with each product multiplied by the corresponding genotype probability of returning at age 5. An important difference between Models A and B is shown by eq. (2): if $p_{AA} = 1$, $p_{Aa} = 0.5$, and $p_{aa} = 0$ (simple additive effects of the alleles), then p_5 will be linearly related to s' as in Model A; otherwise there will be a quadratic relationship between p_5 and s' for Model B.

Predicted Behavior of a Single Cycle Line with Age 5 Inputs

To provide some insight about the population consequences of heritable age at maturity, we first examine a simple model for generation to generation changes in a single cycle line that receives a fixed input A_5 of age 5 spawners each generation, from the previous cycle line. If the total number of spawners in generation t for the cycle line is S_t and the total recruitment from these spawners follows a Ricker curve, then the expected number of spawners that will be present at time $t + 4$ is given by

(3) $S_{t+1} = A_5 + (1 - p_5)\, S_t \exp\{a - F - bS_t\}$

where a and b are Ricker productivity and density-dependence parameters, F is the fishing rate suffered by age 4 recruits in year $t + 4$, and p_5 is the proportion of age 5 progeny produced by S_t. Under the genetic models, p_5 will be an increasing function of A_5/S_t. For most Fraser River sockeye, a is around 1.9 and F has averaged around 1.6–1.9 (exploitation rate 0.8–0.85) since 1948 (Walters and Staley 1987; Cass 1989); the density dependence or carrying capacity parameter b varies greatly among stocks (Cass 1989). According to these parameter estimates, the low cycle lines (for which the bS_t term in eq. (3) is negligible) should be stable or increasing at rates of up to 40% per generation ($a - F = 0.3$–0.4) if fish were not being lost from those lines at differential rates (lower a, higher F, or production of 5s). Stock recruit data generally do not indicate lower a values for the low cycle lines.

Consider the cycle line relative growth rate per generation, S_{t+4}/S_t, predicted by eq. (3). A relative growth rate of 1.0 at some S_t implies that the line is just holding its own. The cycle line will tend to remain around this size if the relative growth rate is decreasing in S_t and will tend to increase (escape depensatory effects) if the relative growth rate increases with increasing S_t. The growth rate will have two components: (1) A_5/S_t, which decreases as the stock size increases, and (2) a "self-reproducing" component $(1 - p_5)\exp\{a - F - bS_t\}$, which will be a decreasing function of S_t for large stock sizes (compensatory effect of bS_t term), but may show more complex behavior when S_t is small depending on how p_5 varies with S_t.

One key prediction is apparent from inspection of eq. (3): if production of 4-type offspring ($1 - p_5$ term) is just proportional to the number of spawners in excess of A_5, then the ratio S_{t+4}/S_t will be an increasing function of S_t for low S_t, and there will not exist any stable equilibrium at low stock size (Fig. 4).

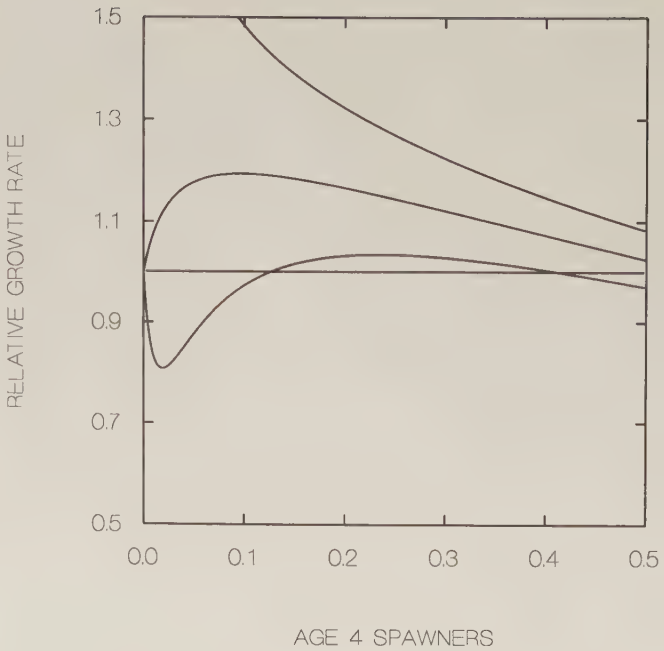

FIG. 4. Relative cycle line growth rate (N_{t+4}/N_t predicted by eq. (3) when a constant number of age 5 fish ($A_5 = 0.03$) is added to the cycle line spawning stock. Top curve shows Ricker model prediction if all recruits return at age 4. Middle curve shows prediction if proportion of recruits at age 4 is just equal to proportion of age 4 spawners. Lower curve shows prediction if proportion of recruits at age 4 is equal to square of proportion of age 4 spawners.

The stock will tend either to decline toward a minimum level equal to A_5 or to escape toward a much higher equilibrium. To understand this prediction, note that if $1 - p_5 = 1 - A_5/S_t$ and if bS_t is small, then S_{t+4}/S_t will be given approximately by $[A_5(1 - \exp\{a - F\}) + S_t]/S_t$, which is increasing in S_t. The inheritance Model A with $i = 1.0$ and Model B with additive phenotypic effects both predict progenal 4-type production proportional to parental 4-type proportion and hence will not produce a stable equilibrium at low stock sizes with a mixture of 4- and 5-type fish unless there is some intergenerational linkage that creates feedback through long-term changes in A_5.

Figure 4 shows a more complex pattern of behavior for S_{t+4}/S_t in relation to S_t, which is possible if $1 - p_5$ varies quadratically with A_5/S_t such that only very low proportions of 4-type offspring are produced until A_5/S_t declines substantially. This pattern involves a low equilibrium stock size at A_5, and the possibility of escaping this low equilibrium if some accident (or reduced fishing) produces a large S_t. Such a pattern is expected for example in Model B if the 4-type allele (a type) is recessive, while the AA and Aa types have a significant probability of returning at age 4.

The only obvious mechanism that we can see for producing a stable equilibrium at low stock size is for the low spawning run to consist entirely of genetically 5-type fish, with incomplete expression ($p_{AA} < 1.0$) so that substantial age 4 returns are produced (as observed) but then consistently lose large numbers of offspring from the cycle line (to age 5 production) so that the line is maintained primarily through recruitment of new 5-types from previous lines (i.e. through the A_5 additions). Considering recent "invasions" by 5-yr-old fish of offcycle lines that were apparently completely extinct (no spawners counted) in stocks such as the Horsefly, Mitchell, Portage, and Scotch creek, perhaps the hypothesis of pure 5-type lines is not

so far fetched as it might initially appear. The 5-type fish may represent "temporal colonists" that initially dominate offcycle lines after extinction events, and it may take a very long time for 4-type fish to reinvade such lines. However, the temporal colonization model cannot explain why the second offcycle lines in DSLL stocks generally have lower proportions of age 5 spawners and offspring than do the first offcycle lines.

Conditions for Equilibrium with Cycle Line Linkages

Equation (3) examines growth rates per generation for single cycle lines, given fixed inputs A_5 of age 5 fish from previous lines. But for an equilibrium to exist among cycle lines at spawning stock sizes S_c, $c = 1, 2, 3, 4$, each cycle line must behave according to

$$(4) \quad S_c = (1 - p_c)\, r_c S_c + p_{c-1} r_{c-1} S_{c-1} S^*$$

where p_c is the proportion of age 5 recruits produced at equilibrium by cycle line c, $c - 1$ is defined as 4 for $c = 1$, r_c is the net productivity of cycle line c (recruits/spawner times survival through fishing) predicted to be $r_c = \exp\{a - F - bS_c\}$ by the Ricker model, and S^* is a relative survival rate from age 4 to age 5 for age 5 recruits. Equation (4) sys simply that the equilibrium number of spawners S_c in each cycle line will equal a contribution $(1 - p_c) r_c S_c$ of 4-yr-olds from the same number of spawners 4 yr previously, plus an addition $p_{c-1} r_{c-1} S_{c-1} S^*$ of 5-yr-olds from the equilibrium number of spawners of the previous cycle line. Equation (4) represents a system of four conditions that an equilibrium must satisfy; the equilibrium may of course be unstable as indicated by the analysis of eq. (3), but eq. (4) can at least be used to infer what combinations of p_c, r_c, and S_c would result in temporary stability of a cyclic dominance situation.

One immediate prediction from eq. (4) is that the equilibrium proportion s_c of age 5 spawners in each cycle line will just be $s_c = 1 - (1 - p_c) r_c S_c / S_c = 1 - (1 - p_c) r_c$. This prediction can be reexpressed as a functional relationship between p_c and s_c; for an equilibrium, p_c must satisfy

$$(5) \quad p_c = 1 + (s_c - 1)/r_c.$$

This requirement for the parent–offspring relationship is quite different than assumed in Model A (eq. (2)), particularly through its implication that the relationship must vary among cycle lines depending on the relative productivities r_c. Thus it would seem that Model A is too simple a relationship to produce stable cyclic dominance; if Model A is empirically correct, then genetic mechanisms alone cannot be repsonsible for persistent, stable cyclic dominance.

Let us assume for a moment that the observed average spawning stocks $S_1 = 1.06$ million, $S_2 = 0.252$, $S_3 = 0.0043$, and $S_4 = 0.0032$ for the Lower Adams River stock do in fact represent equilibrium levels. Then substituting these values into eq. (4), we can solve either for (1) the r_c values needed to produce equilibrium given the average historical proportions of spawners s_c ($s_1 = 0.001$, $s_2 = 0.04$, $s_3 = 0.25$, and $s_4 = 0.02$) or (2) the p_c values needed to produce equilibrium if the Ricker model predictions of r_c are on average correct as predictors of fishing and overall spawning density effects. For $a = 1.9$, $b = 0.25$, and $F = 1.63$, the Adams River r_c values are predicted by the Ricker model to be $r_1 = 1.0$, $r_2 = 1.22$, $r_3 = 1.30$, and $r_4 = 1.30$.

Solving eq. (4) for r_c given the observed Adams River spawning populations and proportions of age 5 spawners, with $S^* = 0.7$, results in $r_1 = 1.012$, $r_2 = 0.97$, $r_3 = 0.77$, and $r_4 = 1.50$. Assuming $S^* = 1$ results in essentially the same values, except $r_4 = 1.34$. These values are clearly inconsistent with the Ricker model predictions; in particular, for an equilibrium the first offcycle needs to be much less productive than predicted by the Ricker model. Either the observed mean spawning stocks do not represent an equilibrium situation or else some mechanism besides genetic effects is causing the r_c values to vary differently among cycle lines than predicted by simple density dependence and equal fishing losses (as expressed in the Ricker model). However, the r_c estimates are extremely sensitive to the assumed average spawning proportions s_c; in particular, small changes in the assumed proportion for $c = 1$ (dominant line), where the estimates are unreliable anyway due to small sampled proportions of 5-yr-olds, lead to large changes in the r_c estimates for the second offcycle. Therefore, this check for equilibrium conditions could be misleading.

Solving eq. (4) for p_c given the observed Adams River spawning populations and Ricker predictions of r_c results in ridiculous p_c values unless S^* is assumed to be very low (eq. (4) has no unique solution for p_c if $S^* = 1$). For example, assuming $S^* = 0.7$ results in $p_1 = 0.97$, $p_2 = 0.75$, $p_3 = -8.66$, and $p_4 = -8.32$. Again, the implication is that either the observed spawning stocks do not represent an equilibrium or else that the r_c values have a more complex pattern than predicted by the Ricker model with equal fishing rate on all cycle lines. In other words, either the Adams is not at equilibrium or else the genetic model implied by eq. (4) and (5) (along with simple Ricker model density dependence in productivity) is not sufficient to explain the observed differences among cycle lines.

There is good evidence that at least the subdominant lines are not at equilibrium in the major DSLL stocks. The subdominant line has shown substantial growth in the Adams and Horsefly stocks, and the Adams subdominant was deliberately fished down (90% exploitation rate) in 1971 (IPSFC 1972). Recognizing this, we recomputed the r_c and p_c equilibrium predictions for the Adams stock, assuming $S_2 = 1.06$ (subdominant equilibrium same as dominant). Treating the historical s_c values as equilibrium values, with $S^* = 0.7$, then resulted in $r_1 = 1.05$, $r_2 = 0.96$, $r_3 = 0.77$, and $r_4 = 1.50$. Thus, changing the assumed equilibrium values of S_c does not alter the conclusion that something besides simple density dependence must be acting differently on the two Adams offcycle lines, unless the historical s_c estimates are nonrepresentative or have been measured incorrectly. Treating the Ricker r_c values as correct, eq. (4) and (5) predict equilibrium (s_c, p_c) combinations of (0.0016, 0.0016), 0.0014, 0.0014), (0.197, 0.383), and (0.469, 0.592) for the $S_2 = 1.06$, $S^* = 0.7$ assumption. These combinations are not at all unrealistic in view of the data from other DSLL stocks, except that the second offcycle line is predicted to have relatively more 5-yr-old spawners than the first offcycle (for all S^* assumptions). An interesting feature of the (s_c, p_c) combinations required for equilibrium is that they represent disproportionately high production of 5-type progeny as s_5 increases; this would be expected under Model B if the 5-type allele is dominant.

From the calculations reported in this section, we conclude that stocks like the Adams are either not at equilibrium in a DSLL cyclic dominance pattern or else that the equilibrium involves variations in productivity among cycle lines (r_c variation) that cannot be explained only by production of 5-type offspring. If the subdominant line is not in fact at equilibrium for stocks such as the Adams, then it remains possible that there

is a genetically produced equilibrium in the offcycles through a mechanism such as the "temporal colonization" model mentioned in the previous section.

Predicted Temporal Behavior with Linkage among Cycle Lines: Deterministic Case

To evaluate how inheritance of 5-type production might influence population dynamics, it is necessary to look beyond single cycle line models such as eq. (3) and equilibrium models such as eq. (4) and examine how transfers of animals among cycle lines (A_5 spawner additions) might vary over time. In this and the following section we use a simple simulation model for that evaluation.

The simulation calculations for each year are organized as follows. First, a total spawning run is composed from 4- and 5-type recruits (split by genotypes *AA*, *Aa*, and *aa* for Model B) to which a constant exploitation rate (equal for all ages, cycle lines, and years) is applied. Fecundity differences between 4- and 5-year-old females (Healey 1987) are assumed small enough to ignore. The parental proportion s_5 (for Model A) or genotypic proportion s' (for Model B) is calculated from the appropriate recruit proportions. The Ricker model as in eq. (3) with $a = 1.9$, $b = 0.25$ is then used to calculate total recruitment R_t at age 4 from the spawning run, and these recruits are apportioned to future years $t + 4$ and $t + 5$ using the parental proportions and either Model A or B. As for eq. (4), age 5 recruits (of whatever genotype) are assigned a relative survival rate S^* to represent the additional year that they spend at sea, so that if the nominal proportion of age 5 recruits from the genetic model is p, the age 4 recruits at $t + 4$ are calculated as $(1 - p)R_t$ and the age 5 recruits at $t + 4$ are calculated as pS^*R_t.

We initialized each simulation with a cyclic escapement pattern in the ratios 1.0:0.1:0.01:0.008 (similar to the Adams River stock), with 5-type fish in the phenotypic percentages 0.1:3.9:26.3:1.8 (again like the Adams). For genetic Model B, these percentages were also assumed to represent the initial genotypic percentage of *AA* fish, and percentages of the other alleles were assigned by assuming Hardy–Weinburg equilibrium (we found the long-term predictions to be insensitive to this initialization procedure for Model B). The relative survival rate S^* for 5-type recruits was taken to be 0.7 as a base assumption, and a variety of other values ranging from 0.1 to 1.5 were tested. The fishing rate F was set high enough to just stabilize the dominant (first) cycle line at its initial value S_1 ($F = a - bS_1$), so that the other cycle lines will increase toward S_1 in the absence of genetic effects tending to maintain the cycle.

The deterministic simulation results for Model A are not encouraging (Fig. 5). Even with very high parent–offspring correlation ($i = 1.0$ in eq. (1)), the model predicts damping of cyclic variation almost as quickly with the inheritance effect as for a population producing pure 4-type offspring. Also, it predicts that the highest percentage of 5-type spawners should be in the second offcycle year and that the 5-type percentages should increase at first but then decline over time due both to $S^* < 1$ and to swamping by 4-types. The basic problems are that (1) the percentage of 5-type spawners is initially not high enough to cause strong enough offspring loss from low cycle lines, (2) losses that do occur are more than balanced in the long run by the higher productivities per spawner predicted for low cycle lines by the Ricker model, and (3) the basic effect is unstable (depensatory) as noted in the previous section, and multigeneration effects on A_5 are not large enough (or consistently in the right direction) to provide stability. Setting $i < 1$ so as to flood more 5-types into the low cycle lines does not improve matters; it causes decline in the dominant line, but actually increases the rebuilding rates in the offcycle lines by returning more 4-types from the 5-types dumped onto those lines. Increasing S^* so as to prevent loss of 5-types causes a slightly stronger suppression of offcycles in the short term (first few generations), but then enhances rebuilding rates in the long term.

Figure 5 also shows encouraging results for a variation of Model A, where we assumed that the proportion of age 4 offspring is on average equal to the square of the proportion of age 4 parents (age 4 production a "recessive" trait). This model will produce persistent cycles if the marine survival parameter S^* is chosen carefully: low values ($S^* < 0.5$) result in quick disappearance of 5-types, while high values ($S^* > 0.8$) result in 5-types quickly taking over the population entirely (and breaking down any dominance pattern).

We tried many simulations with Model B, using different assumptions about the age 5 return proportions p_{AA}, p_{Aa}, and p_{aa}. We could find no combination of values that would result in persistent cyclic dominance. Intermediate values (>0.0 but <1.0) for any two of the three proportions lead to a "spreading" of whichever allele codes mainly for age 4 returns across cycle lines, leading to enhanced rebuilding of the offcycle lines. Only by assuming a strong dominance system with $p_{AA} = 1$ but $p_{Aa} = p_{aa} = 0$ (*a* allele dominant for return at either age 4 or age 5) could we get any persistent effect on the offcycle lines, and even in these cases we had to assume high relative survival rates ($S^* > 0.9$) to prevent the *A*-type allele from disappearing within 10–15 cycles. In the case where the *a* allele codes for return at age 5, the single cycle line model had suggested the possibility of a stable equilibrium due to quadratic variation in $1 - p_5$ (Fig. 3; Model A quadratic variant in Fig. 5); however, when considered over all cycle lines, any stability is eventually lost due to transmission of 4-type alleles to the offcycle lines in the form of *Aa* heterozygous fish.

We could only find one simple genetic arrangement that would produce stable DSLL cycles in the deterministic case, with anything like the observed proportions of age 5 spawners: a strong dominance pattern for age 4 returns but not age 5 returns (e.g. $p_{AA} = 0.5$, $p_{Aa} = p_{aa} = 0$), in conjunction with initial gene frequencies $g_{AA} = 1$, $g_{Aa} = g_{aa} = 0$ for the offcycle lines. This arrangement corresponds to having the offcycle lines consist entirely of genetic 5-type fish that have "colonized" the line (due to $p_{AA} < 1.0$) after an extinction event (such as the Hells Gate disaster). It will produce nicely persistent cycles (Fig. 6) for a wide range of p_{AA} values provided $S^* < 1.0$. If S^* is 1.0 or larger, the offcycle lines eventually (after 100+ yr) begin to rebuild.

The results presented so far are robust to two complications that might be added to make the genetic model more realistic. First, it is conceivable that there is a heterozygote advantage for *Aa* genotypes, such that these types return only at age 4 and are most numerous in the dominant cycle lines; matings among these types would then inevitably produce a steady flux of homozygous 5-types to the offcycles. The trouble with this mechanism is that it is only a way of fixing A_5 of eq. (3) at a level largely independent of offcycle abundance; the presence in offcycle lines of any 4-types to mate with the 5-types will result in more heterozygotes that will then return at higher rates

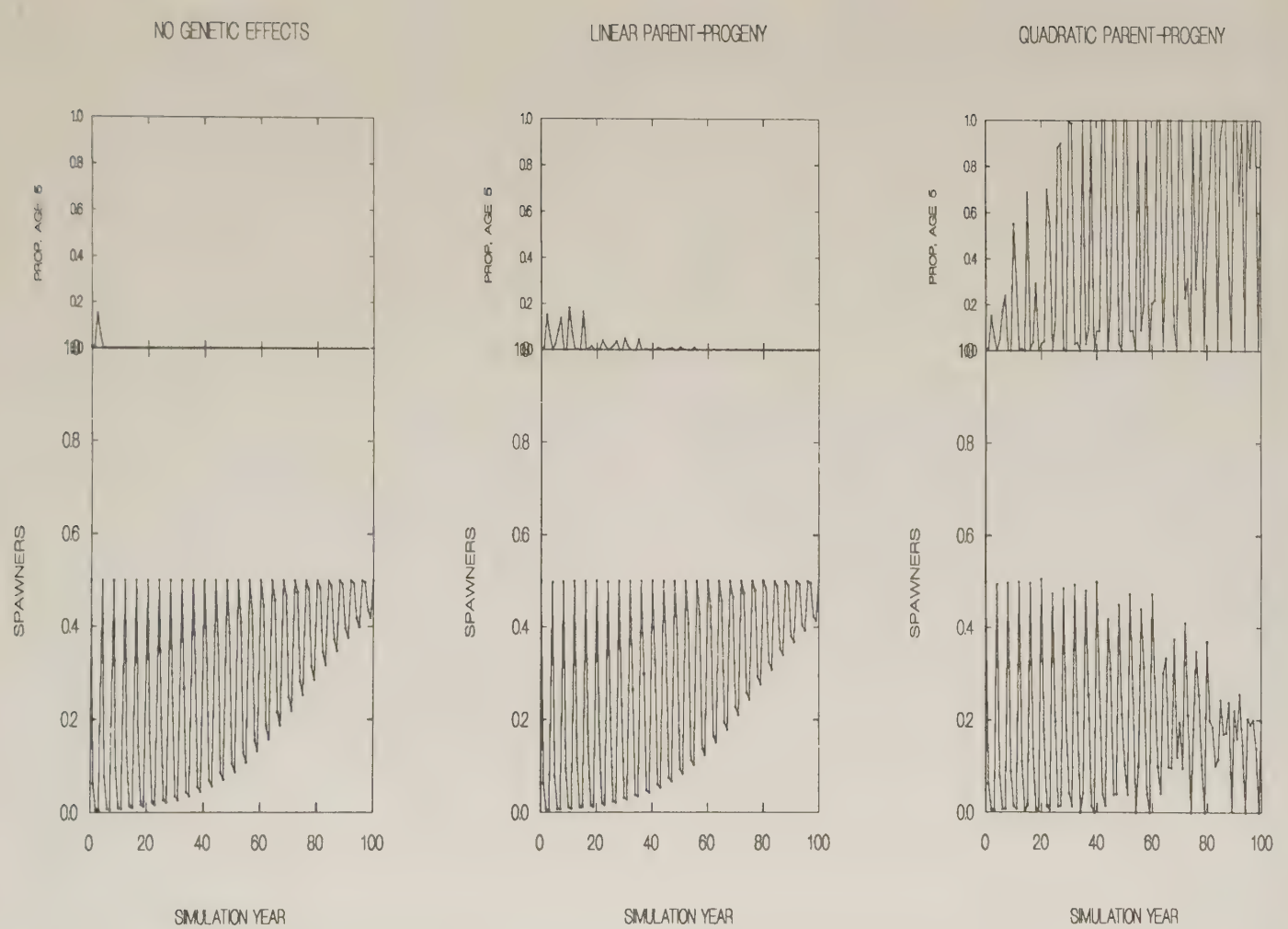

FIG. 5. Simulations of long-term population change using a Ricker model and equal harvest rates (80%) in all years. Left panel: all recruits at age 4; middle panel: proportion of age 4 recruits equal to proportion of age 4 spawners, initial spawner proportions equal to Adams historical average (Fig. 1); right panel: proportion of age 4 recruits equal to square of proportion of age 4 spawners, initial spawner proportions again from Adams historical average.

and eventually dilute the effects of the 5-type additions. Second, there may be assortative mating, based on body size or run timing differences between age 4 and 5 fish. If the assortment favors like–like matings, it will reduce the effect on off-cycles of having 5-types added to them. If the assortment favors unlike matings (e.g. selection of larger 5-type males by 4-type females), it could increase the loss of 4-types from any line (in the form of heterozygous offspring if these offspring tend to return at age 5), but such losses would become progressively less important if the number of 4-types started to increase for any reason.

Predicted Behavior with Linkage and Stochastic Effects

Substantially different results are obtained from the simulations for both Models A and B when realistic levels of random "environmental" variation are included in the stock–recruitment calculation. We included random effects as normally distributed values with mean 0.0 and no temproal autocorrelation added to the exponent of the Ricker model (i.e. log-normal random effects), with all genotypes equally affected. Based on estimates in Walters and Staley (1987) and Cass (1989), we assumed a variance of 0.8 for the effects.

Figure 7 shows a 200-yr simulation of inheritance Model A, with $i = 1.0$ and $S^* = 0.9$ (for lower S^*, the 5-type disappears rapidly as in the deterministic simulations). The striking feature of this simulation is that the dominance pattern is much more persistent than in deterministic simulations and even reappears after breaking down part way through the run. The proportion of 5-type fish does eventually decline, but much more slowly than in the deterministic simulations; in effect, the simulation creates a modest "bet hedging" advantage to producing offspring with mixed age at return.

Similar results to Fig. 7 are obtained with Model B for the same parameter combinations (dominance patterns) that produce the most persistent cycles in the deterministic simulations. Random variation helps to maintain strong cycles (and create them when they are not present initially) and favors persistence of 5-type alleles that would otherwise disappear slowly due to $S^* < 1.0$. Additionally, the stochastic simulations will occasionally result in "flips" in the dominance pattern, with new cycle years becoming dominant and/or an entirely different pattern persisting for some cycles. For example, a "Nadina" pattern (see Fig. 1) will appear and persist for up to 30 cycles, involving alternating dominant and weak cycle lines on a 2-yr cycle. Another common and somewhat persistent pattern is like

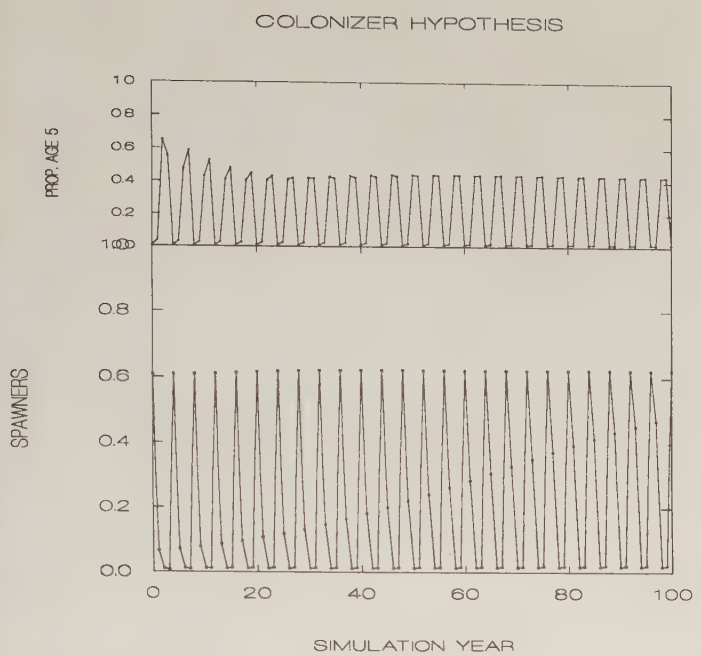

FIG. 6. Simulation of the "temporal colonizer" hypothesis. Offcycle lines assumed to consist initially of 100% genetic 5-type fish, of which 50% will show age 4 phenotype. All 4-type and heterozygote fish assumed to return only at age 4.

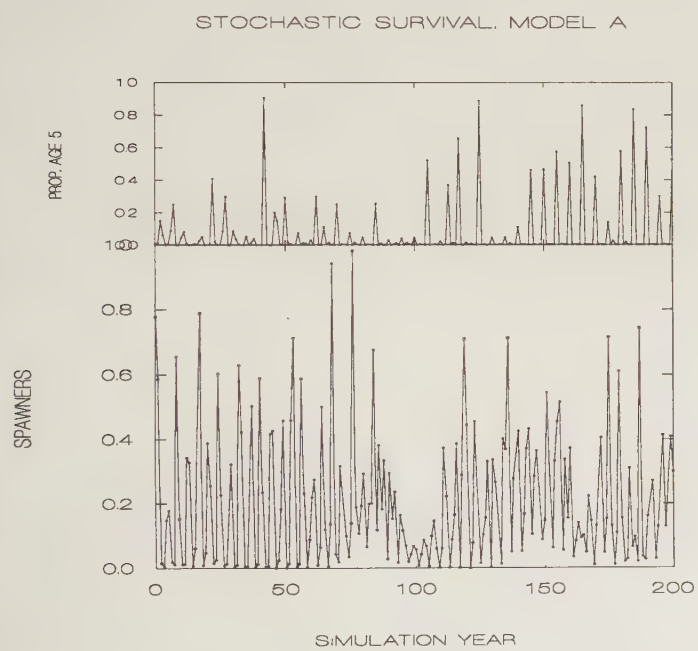

FIG. 7. Long-term simulation of genetic Model A (progeny proportion age 5 equal to parent proportion age 5) in a stochastic environment. Compare with middle panel of Fig. 5.

the Chilko stock, with a low line followed by progressively larger lines so that the last cycle line in each 4-yr cycle is the largest. However, flips in dominance to Nechako- and Chilko-like patterns are also predicted by stochastic models containing no linkages among cycles (i.e. stocks with only age 4 returns), providing fishing rates are very high on all cycle lines so that recovery is slow after accidental years of low survival.

Management Implications

Based on the data and modelling results presented above, we suspect that genetic or maternal effects on production of age 5 offspring are at least partly responsible for maintaining or slowing the recovery of offcycle lines in Fraser sockeye. Following complete extinction of lines, recolonization by 5-type fish could result in low cycle lines that persist indefinitely at low levels, losing large percentages of their offspring production to larger cycle lines.

The Canadian Department of Fisheries and Oceans is currently evaluating various experimental options for attempting to rebuild offcycle lines through harvest rate reductions and enhancement (Collie et al. 1990; Welch and Noakes 1990; C. Wood, pers. comm.). Projections of stock rebuilding rates for this evaluation have been based mainly on Ricker models and Larkin's (1971) revision of the Ricker model to account for among-cycle line regression effects on recruitment per spawner, and either ignore or improperly account for heritable variations in age at maturity. The projections of rebuilding rate under various harvest rate reductions may be too optimistic. Likewise, enhancement planning has not accounted for the risk that some technologies (like spawning channels) may favor the "wrong" genotypes; a technology that inadvertently favors production of 5-type fish may lead in the long term to reduced productivity in all cycle lines, even if it is directed initially only at offcycle lines.

Our results should not be interpreted as a condemnation of proposals for experiments to rebuild offcycle lines. Such experiments could provide strong tests of the genetic models, if they include (1) more precise monitoring of parent and progeny age composition for offcycle lines, (2) enhancement experiments involving controlled matings of the age phenotypes, while following progenal production and characteristics from these matings by means of tagging, and (3) tagging and recovery of outmigrant smolts from the offcycle lines (Fennell, Horsefly, etc.) that have recently appeared again after apparent extinction, so as to more precisely measure patterns in age at return for these very special fish that have recolonized the offcycles. There is a grand opportunity through these experiments to provide more information about the mechanisms that control age at maturity in sockeye salmon in general, and the results might help in developing better understanding and forecasting for sockeye salmon throughout the Pacific region.

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Dynamics and Species Interactions in the Commercial Fishery of the Red Lakes, Minnesota[1]

Donald L. Pereira, Yosef Cohen, and George R. Spangler

Department of Fisheries and Wildlife, University of Minnesota, St. Paul, MN 55108, USA

Pereira, D. L., Y. Cohen, and G. R. Spangler. 1992. Dynamics and species interactions in the commercial fishery of the Red Lakes, Minnesota. Can. J. Fish. Aquat. Sci. 49: 293–302.

The Red Lakes, Minnesota, have supported a commercial fishery for walleye (*Stizostedion vitreum vitreum*) and yellow perch (*Perca flavescens*) since 1917. Since 1972, harvests indicate increased variance in recruitment of percids and an increase in biomass of freshwater drum (*Aplodinotus grunniens*). We subjected commercial catch-per-unit-effort (CPUE) records of walleye, yellow perch, and animal feed (composed primarily of drum) to spectral analysis. Estimated power spectra displayed peaks at 8.5, 10, and 17 yr for walleye, yellow perch, and animal feed, respectively. Walleye and yellow perch CPUE time series were significantly coherent at periods ranging from 5 to 10 yr, confirming the predator–prey relationship and common recruitment patterns of these two species. Interpretation of coherency between drum and the two percid species required knowledge of the age distribution of freshwater drum. The apparent exponential increase in drum biomass as indicated by commercial CPUE is primarily due to strong year classes in 1955, 1970, and 1983. While drum recruitment and growth are consistent with the periodicity in the autospectrum, we find little evidence from coherencies that drum recruitment is related to the dynamics of wlleye or perch populations.

Depuis 1917, les lacs Red de l'État du Minnesota ont alimenté une pêche commerciale du doré jaune (*Stizostedion vitreum vitreum*) et de la perchaude (*Perca flavescens*). Par contre, depuis 1972, les captures révèlent une plus grande variance du recrutement de percidés et une augmentation de la biomasse du malachigan (*Aplodinotus grunniens*). Les auteurs ont effectué une analyse spectrale de données sur les prises par unité d'effort (PUE) de doré jaune et de perchaude, ainsi que sur des aliments d'animaux composés en grande partie de chair de malachigan. Les spectres de puissance estimatifs ont montré des pointes à 8,5, 10 et 17 ans dans le cas du doré jaune, de la perchaude et des aliments d'animaux, respectivement. Les séries temporelles de données sur les PUE étaient nettement cohérentes selon des périodes variant de 5 à 10 ans et étayent la relation prédateur–proie et les régimes communs de recrutement de ces deux espèces. L'interprétation de la cohérence entre le malachigan et les deux espèces de percidés nécessitait des connaissances sur la distribution des âges du malachigan. L'augmentation exponentielle apparente de la biomasse de malachigan, telle que révélée par les PUE commerciales, est en grande partie le résultat de l'apport d'importantes classes d'âges en 1955, 1970 et 1983. Même si le recrutement et le taux de croissance du malachigan concordent avec la périodicité de l'autospectre, les auteurs n'ont relevé que de faibles preuves dans les cohérences que le recrutement du malachigan est lié à la dynamique des populations de doré jaune et de perchaude.

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The Red Lakes in northern Minnesota consist of two oval basins totalling 116 876 ha with a mean depth of 5.5 m and a maximum depth of 10.7 m. The lakes are isothermal during the ice-free season due to wind mixing and are moderately fertile with a morphoedaphic index (Ryder 1965) of 40. Knapp (1960) provides additional limnological detail. The Red Lake Band of Chippewa Indians has operated a commercial fishery since 1917 (Smith and Krefting 1954). Primary species include the walleye (*Stizostedion vitreum vitreum*) and yellow perch (*Perca flavescens*). Species of secondary commercial value include lake whitefish (*Coregonus clupeaformis*), northern pike (*Esox lucius*), and goldeye (*Hiodon alosoides*).

Changes have occurred in the Red Lakes fishery since the most recent published report by Smith (1977). Increased harvest of freshwater drum (*Aplodinotus grunniens*) following low harvest of both walleye and perch around 1972 is the primary indicator of successional change in the fish community. Since 1970, percid recruitment has shown considerable variation, and freshwater drum, a species with low market value, dominates the total fish harvest in most years. The walleye catch-per-unit-effort (CPUE) time series shows patterns similar to other lakes which have supported intensive commercial fisheries (Regier et al. 1969; Payer et al. 1987).

Our objective was to explore the structure of the commercial fishery system of the Red Lakes and identify potential species linkages that may explain recent successional changes in the fish community. We applied multivariate time series analysis in the frequency domain to commercial CPUE data. Species analyzed include walleye, yellow perch, and freshwater drum. Time series may be analyzed in time (Box and Jenkins 1976) and frequency domains (Bloomfield 1976). Applications of these methodologies include fisheries (Saila et al. 1980) and ecological data (Platt and Denman 1975). A common application in fisheries is forecasting CPUE or yield (Mendelssohn 1980; Roff 1983; Jeffries et al. 1989), which is more efficiently

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achieved with analysis in the time domain. Multispecies fisheries research and management (May et al. 1979; May 1984; Daan 1987; Christie et al. 1987; Carpenter 1988) provide an impetus for multivariate extensions of time series analysis. Recent multivariate applications in the time domain include forecasting fishery dynamics and examination of community structure (Mendelssohn and Cury 1987; Cohen and Stone 1987; Stone and Cohen 1990). Cohen et al. (1987) also illustrated the utility of frequency domain analysis for exploring perturbations to fish community structure. Limited historical evaluation of freshwater drum in the Red Lakes motivates the use of ancillary data. We therefore report on recent assessment of freshwater drum age distribution and growth which allows more detailed interpretation of time series analysis.

Data

The commercial fishery on the Red Lakes is cooperatively owned and operated by the Red Lake Band of Chippewa Indians. Fishing is almost exclusively with 8.9-cm (stretched mesh) gill nets. Historical, commercial fishing operations as well as general ecology of the Red Lakes ecosystem are detailed in Smith and Krefting (1954), Smith and Pycha (1961), Smith (1977), and references therein. The fishery generally begins in June and ends upon reaching a quota of approximately 306 000 kg of walleye and northern pike. During years of high fishing success, the quota may be increased to 351 360 kg. During years of low CPUE, the fishery may cease operations before filling the quota. Whether or not the fishery operates on any particular day during the regular season is at the discretion of the fishery manager who bases decisions partly on market supply and demand. If the quota is filled during the summer, a fall fishery may resume in October and November where the primary target is lake whitefish, with incidental catch of other species. We define fishing effort as the number of individual fisher's catches recorded each day in the cooperative fishery. Thus, one unit of effort constitutes a single fishing event by a single, registered fisher. We did not deem it necessary to quantify effort as a linear measure of gill nets deployed, nor was it necessary to consider adjustment from cotton to nylon gill nets. Any gains in resolution from these factors would be minimal when compared with the variability in the fishery system. We totalled effort and harvest (kilograms) annually by species and tallied CPUE (average kilograms harvested per single fishing event) from 1938 through 1988 (Table 1).

TABLE 1. CPUE (kilograms per fishing event) of walleye, yellow perch, and animal feed, 1938–88.

Year	Walleye	Yellow perch	Animal feed
1938	102.59	38.26	3.31
1939	68.51	23.60	2.13
1940	80.76	18.12	2.26
1941	76.77	8.15	1.30
1942	48.58	8.53	0.93
1943	37.12	9.17	5.30
1944	76.60	27.38	3.03
1945	55.01	20.33	2.45
1946	54.90	20.31	1.89
1947	52.67	18.11	2.37
1948	61.50	16.64	2.38
1949	44.51	12.51	2.78
1950	20.06	4.16	1.27
1951	29.84	6.00	1.10
1952	57.60	34.63	2.86
1953	52.15	44.02	0.91
1954	53.57	41.49	0.87
1955	45.93	24.47	1.51
1956	39.23	11.50	1.24
1957	45.02	14.53	1.84
1958	32.28	9.77	3.39
1959	21.44	12.04	13.08
1960	31.57	11.21	12.14
1961	77.54	19.04	9.87
1962	71.81	18.18	5.96
1963	26.86	17.92	10.08
1964	37.51	13.85	9.81
1965	26.38	20.59	11.07
1966	61.81	22.06	7.88
1967	103.17	32.81	9.02
1968	63.45	51.44	7.87
1969	38.62	26.57	8.84
1970	48.40	13.15	9.98
1971	24.80	6.65	7.92
1972	8.90	0.97	8.70
1973	9.41	1.45	14.11
1974	6.60	7.95	16.75
1975	15.92	11.86	14.75
1976	17.64	30.94	27.87
1977	36.29	32.94	27.55
1978	37.95	32.31	45.71
1979	10.36	42.24	61.32
1980	5.94	20.27	70.45
1981	7.20	13.82	66.82
1982	15.17	9.56	49.10
1983	34.48	10.54	43.64
1984	35.76	5.07	39.63
1985	30.73	8.20	34.90
1986	21.06	11.83	69.82
1987	26.92	7.52	50.32
1988	53.04	3.94	44.45

Methods

We assumed that CPUE is an index of relative abundance (Peterman and Steer 1981; Bannerot and Austin 1983). Smith and Krefting (1954) supported this assumption for the Red Lakes commercial fishery.

Spectral analysis is the primary tool used for describing time series in the frequency domain. It employs the Fourier transform to fit a sum of sine-cosine functions of varying amplitude and frequencies to a time series (Bloomfield 1976). Coherency is a function calculated from the cross-spectrum of two time series and their autospectra (Priestley 1981). Simply stated, the squared coherency is a measure of linear correlation between two components of a bivariate process (e.g. that derived from two time series) at a particular frequency, or period. For example, in a fishery harvesting two species with unique but consistent seasonal patterns in CPUE, coherence will be high at a period of 1 yr (i.e. one fishing season). Prior to the Fourier transform, we applied a cosine bell taper to 10% of the data on each end of the series. Tapering reduces leakage from strong signals (i.e. those with large amplitudes) into adjacent frequencies (Bloomfield 1976). Also, after computing the fast Fourier transforms for each series, we applied a triangular smoothing or spectral window to the periodograms. Smoothing is essential for statistically consistent estimates of the autospectra and cross-spectra as well as enabling efficient visual examination of the spectra (Bloomfield 1976; Priestly 1981). We chose a triangular spectral window based on computational

simplicity as well as absence of leakage effects from sidelobes (Bloomfield 1976). Finally, we calculated partial squared coherencies for all pairs of series. Partial coherencies are analogous to partial correlations and will remove the potential masking effect arising if the two series are mutually coherent with a third series (Cohen et al. 1987). We calculated values corresponding to 5 and 10% significance for squared partial coherencies according to Priestley (1981). We performed all computation for coherency analysis, including the Fourier transforms, with the Mathematica software package (Wolfram 1988) on a Macintosh microcomputer.

We established commercial fishery age structure and size-at-age of freshwater drum. Freshwater drum were sampled in the 1989 commercial fishery for length and age. We used saggital otoliths to determine age according to the methods of Cunningham (1988).

Results

Preprocessing of Commercial Fishery Data

We selected catches of walleye, yellow perch, and animal feed for analysis. From 1938 through 1988, these groups accounted for 89% of the total harvest (Fig. 1). The highest was 99% in 1984; the minimum was 43% in 1972 when the harvest of percids had declined prior to the increase in drum biomass. Animal feed consists of species and undersized fishes which are undesirable for human consumption. This has primarily consisted of common white sucker (*Catostomus commersoni*) and freshwater drum, with freshwater drum making up a high percentage of animal feed since the mid-1970's. In 1987 and 1988, freshwater drum accounted for 85 and 81% of the recorded animal feed harvest, respectively.

The sqaure root transformation (Weisberg 1980)

$$(1)\quad y_{it} = \sqrt{1 + x_{it}}$$

where x_{it} is the CPUE of series i, $i = 1, 2, 3$, at time t, $t = 1, 2, \ldots, 51$, was applied to decrease the heterogeneity of variance. To reduce nonstationarity, all series were detrended as

$$(2)\quad z_{it} = y_{it} - a - b \cdot t$$

where y_{it} is the square root transformed CPUE of series i, $i = 1, 2, 3$, at time t, $t = 1, 2, \ldots, 51$, and a and b represent least squares coefficients from the regression of y_{it} against time, t, (Fig. 2). The values for z_{it} therefore are equivalent to the residuals from the least squares line. Linear detrending was adequate for walleye and yellow perch and the models fit for these series, respectively, are

$$(3)\quad z_{it} = y_{it} - 8.2 - 0.076 \cdot t$$

$$(4)\quad z_{it} = y_{it} - 4.5 - 0.018 \cdot t.$$

We fit the following quadratic model and used it to detrend the animal feed series:

$$(5)\quad z_{it} = y_{it} - 1.6 + 0.053 \cdot t - 0.004 \cdot t^2.$$

Single Species Spectral Analysis

We qualitatively evaluated three levels of smoothing which included 5, 9, and 13 periodogram ordinates (Fig. 3). Nine ordinates provided an intermediate, suitable degree of smoothing and was therefore selected for calculation of coherencies. All autopower spectra for the three CPUE series had dominant peaks at 9, 10, and 17 yr, respectively, for walleye, yellow perch, and animal feed. Bias in estimating the spectra may affect the precise periods of these peaks. Generally, bias will increase and variance will decrease with degree of smoothing used to estimate the spectrum (Priestley 1981). Also, in all three power spectra, features at 3- to 5-yr periods are not strongly represented due to the degree of smoothing. Also the dominant periodicity masks adjacent ones with smaller amplitudes. However, cycles corresponding to the dominant periods for all series are readily apparent in the preprocessed data (Fig. 2). For the percid species, especially walleye, the fishery has historically harvested three age classes; in recent years, harvests have consisted of two or even one age class. This change in age structure may also entail a change in the frequency of occurrence and size of dominant year classes (i.e. increased variation in recruitment). Faster turnover of the stock through compensatory mechanisms would cause a gradual change in the dominant period. This process would explain the broad, dome shape of the autospectra.

Multispecies Coherency Analysis

Partial squared coherency of walleye and yellow perch displays a distinct peak at a period of 3 yr. A broad range of coherence at periods from 4 through 13 yr follows this minor peak (Fig. 4). We may anticipate this result from the extensive body of literature describing the important predator–prey relationship between these two species. In addition, their life histories share common factors such as spring temperature patterns which may affect survival of egg and larval stages. Walleye and animal feed show significant coherency at a period of 2 yr followed by a peak extending from 4.5 through 6 yr. Yellow perch and animal feed have a narrow peak of significant coherence between 2.5 and 3 yr, and a minor peak around 5 yr which is not significant. All autospectra revealed very little detail at periods which were less than that corresponding to the maximum power value. Walleye and yellow perch both show minor features between 4- and 5-yr periods (Fig. 3). However, since coherency is the linear correlation between two series at a particular frequency, the magnitude (i.e. amplitude) of the autospectra will not affect the estimate of coherency. The cycle of one or both series at a particular period may have small amplitude but still show high correlation with other series. Frequency domain analysis is therefore sensitive to subtle cycles in biological data, and patterns in coherency should not be interpreted independently of the autospectra.

Age Distribution of Freshwater Drum

Results from aging drum are listed in Table 2. In comparison with other populations, drum in Red Lakes exhibit slow growth (Bur 1984). Since 1975 the animal feed yield has consisted almost entirely of the 1970 year class, and (latterly) the 1983 year class of drum (Fig. 1). A small number of older drum currently live in the Red Lakes. However, these larger fish are relatively invulnerable to the commercial gill nets and are seldom encountered during random sampling in the fishery. To obtain additional information on age distribution and recruitment, we purchased fish over 2 kg from fishers (Table 3). Although this sample size is small, it indicates that recruitment of the 1955 year class may explain the increase in harvests experienced in the late 1950's. Also, recruitment of drum is

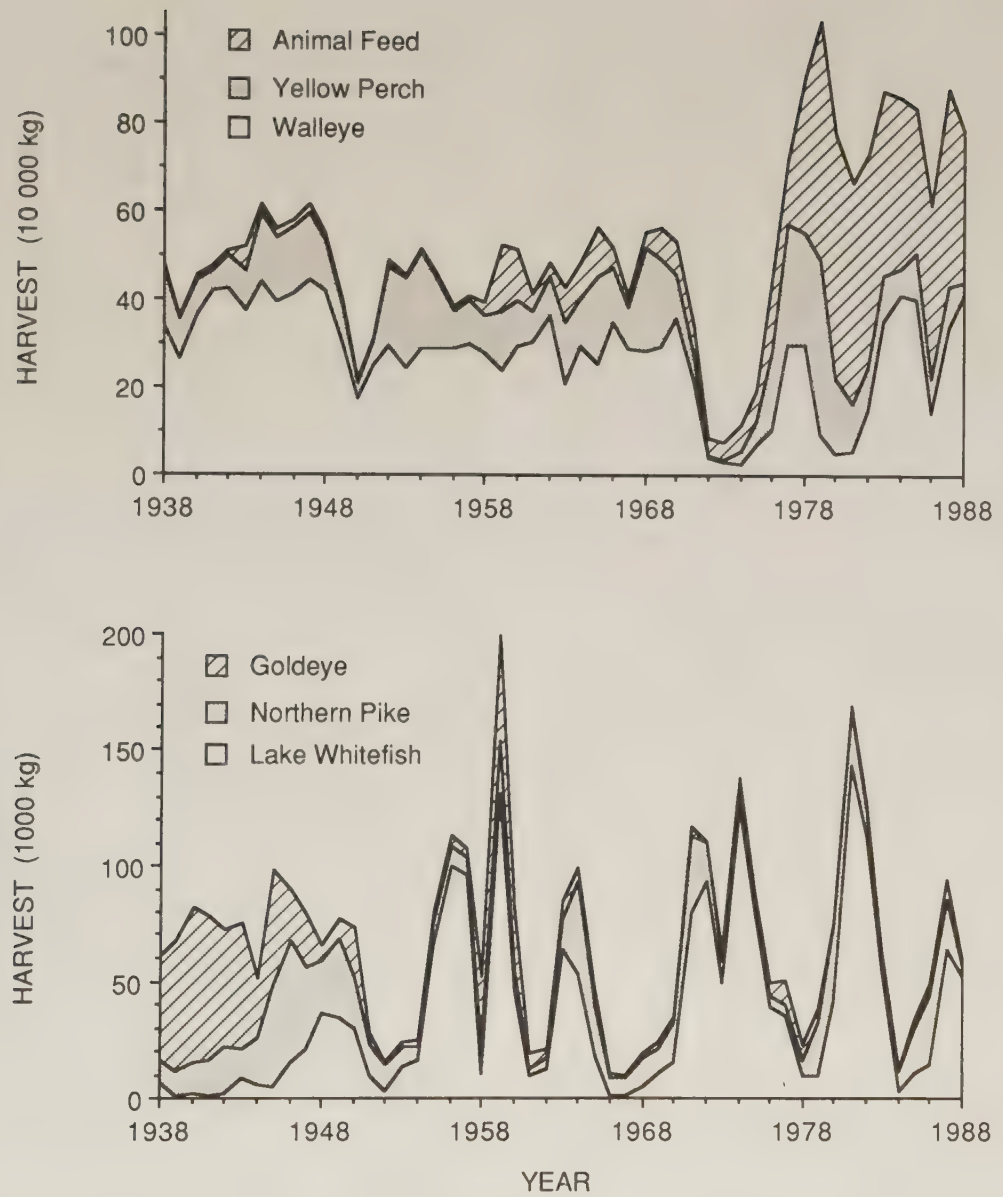

FIG. 1. Species composition of the Red lakes commercial fishery, expressed as kilograms harvested per year, 1938–88.

quite episodic, with long periods (e.g. 1956–69 and 1971–82) in which there is little or no recruitment. This characteristic of drum recruitment is consistent with the large dominant period identified in the autospectra for animal feed (Fig. 2). We also qualitatively explored the relationship between thermal history and year class formation of drum. Recruitment may be associated with a warm growing season; a relationship with winter temperature is less apparent (Fig. 5). Biotic factors affecting survival of young-of-the-year drum may have suppressed recruitment during the warm summers of 1941 and 1976.

Discussion

Our objective was to apply time series methods to develop hypotheses pertaining to successional changes and potential species interactions in the Red Lakes fish community. This discussion will consider how mechanisms and interactions already reported for these species may relate to our results. We will also discuss ancillary data and information from recent assessment of the Red Lakes fish community and identify additional research needs. The methods we present assume that commercial CPUE is an index of abundance, and therefore will be influenced by recruitment variability. Events in the early life history of fishes may be key determinants of year class strength (Blaxter 1974; Shepherd and Cushing 1980; Houde 1987; Miller et al. 1988; Bailey and Houde 1989). Survival during the first year of life is highly variable in many percid species (Craig 1987). Since these findings also apply to Red Lakes fishes, we discuss results in the context of potentially important early life history events.

Walleye and Yellow Perch Interaction

The high coherency we report between walleye and yellow perch is consistent with their predator–prey relationship in Minnesota lakes similar to the Red Lakes (Maloney and Johnson 1957; Swenson and Smith 1976). In the Red Lakes, MN, Smith (1977) and co-workers have documented a strong correlation

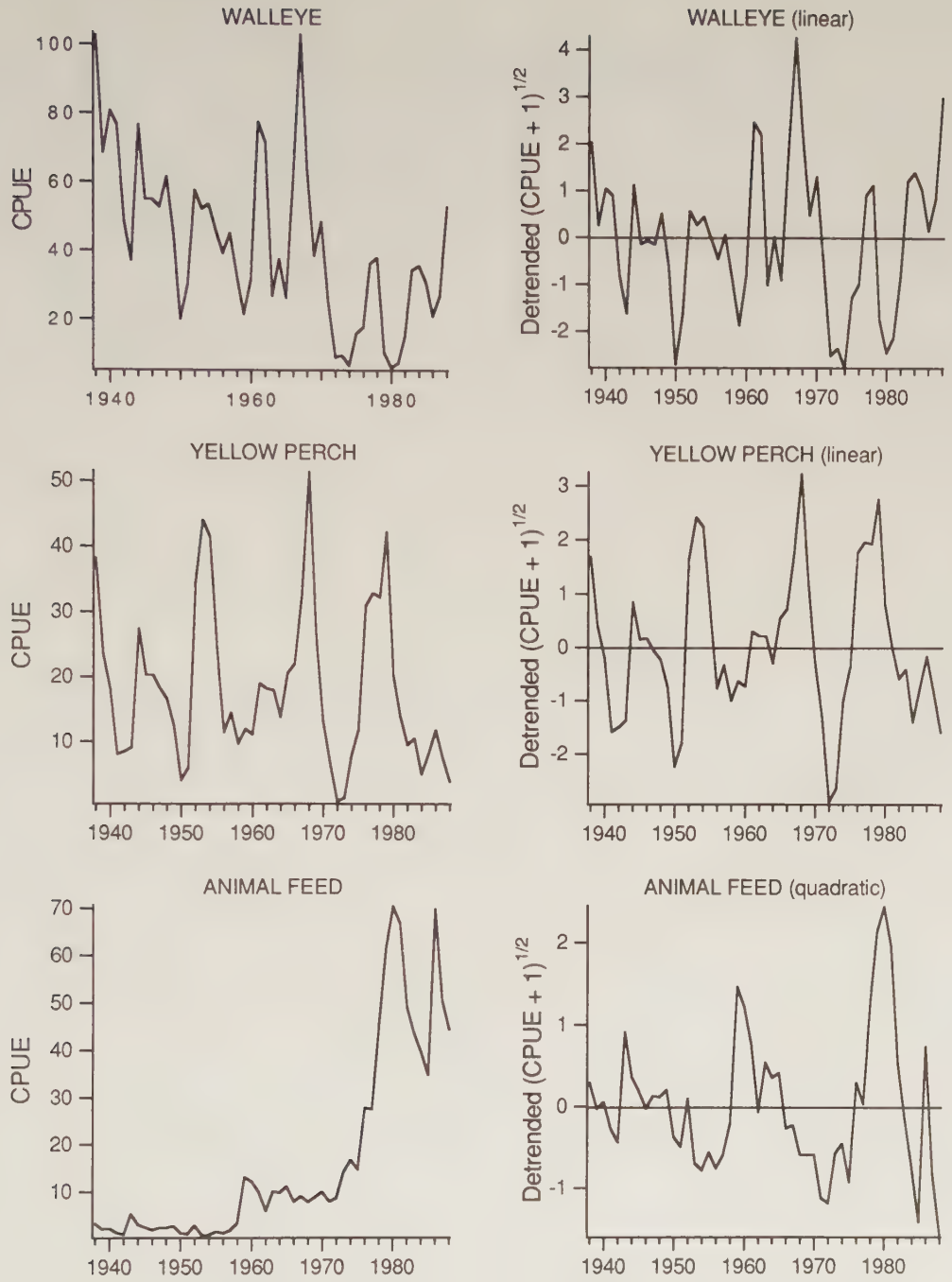

FIG. 2. Raw and detrended CPUE data for walleye, yellow perch, and animal feed. Left panels are raw (untransformed) CPUE as kilograms per fishing event. Right panels are transformed, detrended CPUE.

between year class strength of walleye and yellow perch. They also stated that spring climate patterns affect both species similarly. In contrast, Heyerdahl and Smith (1971) found no relationship in the Red Lakes between perch year class strength and predator (i.e. walleye) abundance. A suite of factors in addition to walleye predation may affect perch dynamics (e.g. larval perch exist in the limnetic zone for a longer period than walleye and their predatory gauntlet is probably more extensive). To explain the coherency reported here, we hypothesize that availability of perch in the Red Lakes as a forage item may positively influence survival of larval and/or juvenile walleye. A test of this hypothesis will require information detailing the spatial and temporal relationships of larval walleye and yellow perch. In many studies on these species, sampling was initiated at the postlarval or juvenile stage (Maloney and Johnson 1957; Ney and Smith 1975). In Oneida Lake over a 3-yr period, Houde (1967), describing the food habits of recently hatched walleye, suggested that perch may provide an important food source and buffer walleye cannibalism. This has been supported by Chevalier (1973). Also in Oneida Lake, Noble (1972) reported a positive correlation between larval perch density and intensity of larval walleye predation on perch. Walleye juveniles grow faster when feeding on fish as compared with invertebrates (Forney 1966), and it is reasonable to suggest that this diet condition may be even more critical for recently hatched larvae.

In contrast with Oneida Lake, Smith and Pycha (1960) found no relationship between growth of juvenile walleye and yellow perch utilization in the Red Lakes. They also reported consid-

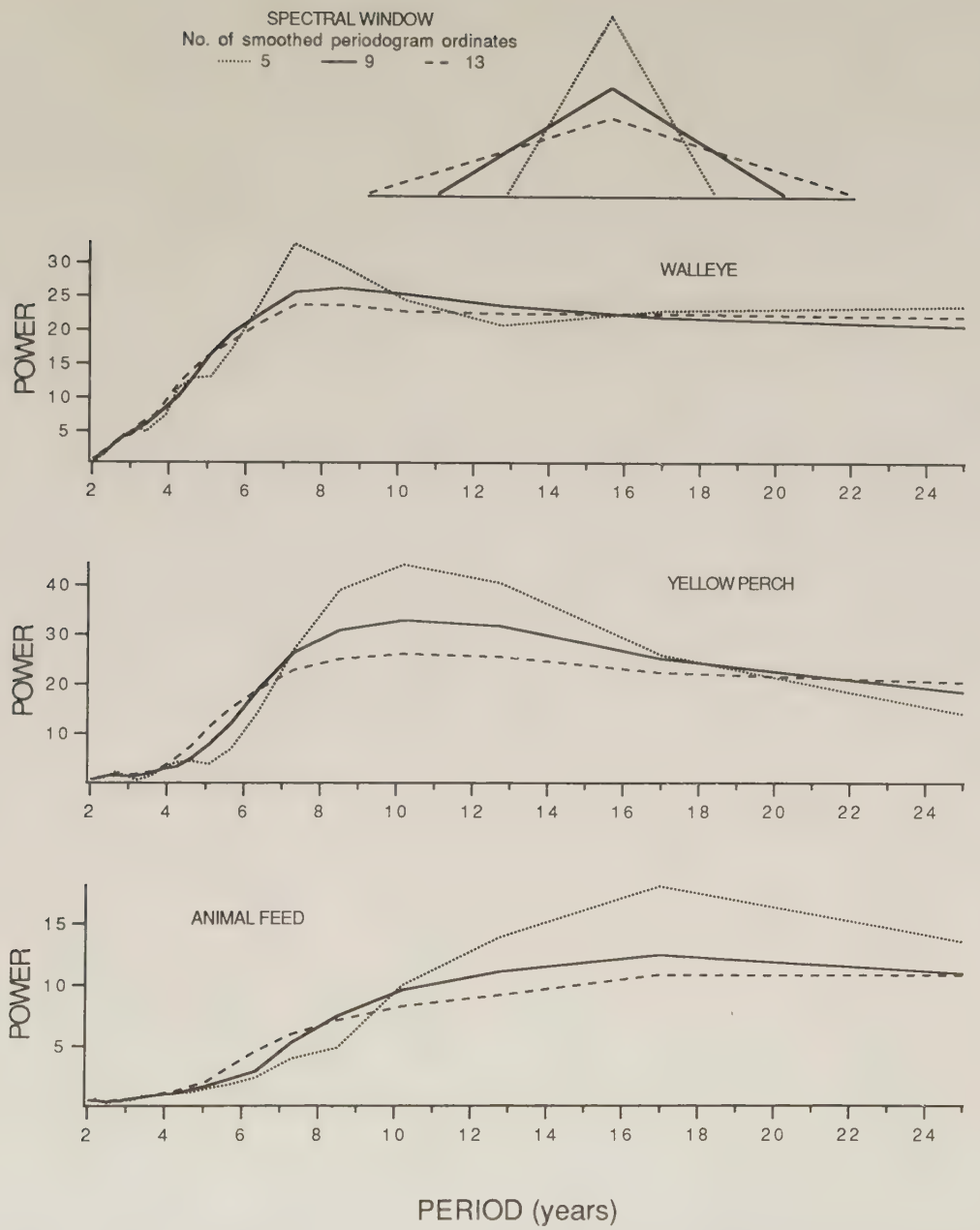

FIG. 3. Autospectra for walleye, yellow perch, and animal feed. The top graph shows the weights for the spectral window which is used to smooth the periodogram. The power spectra denoted by the solid line (i.e. smoothing nine periodogram ordinates) were used for calculation of coherency.

erable utilization of other species by juvenile walleye, notably spottail shiner (*Notropis hudsonius*). Apparent discrepancies between lakes may be resolved if the importance of yellow perch as a prey item for walleye is evaluated at the larval as well as the juvenile stage. Houde (1987) argued that recruitment variability may be due to factors operating on either larvae or juveniles; he also stated that stage-specific growth rate analysis may provide important information for explaining recruitment variability.

Percid Recruitment Patterns and Fishery Effects

Coherency of walleye and yellow perch was significant over a range of periods extending from 3.5 to over 10 yr. Changes in species dynamics over time may explain this result. Smith (1977) reported that walleye and yellow perch showed similar age distributions in the commercial fishery from 1949 to 1964. Both species were represented primarily by age classes IV, V, VI, and VII. However, the recruitment patterns of these species have apparently changed since the early 1970's; the fishery is currently characterized as "boom and bust" and depends on fewer year classes. Therefore, as recruitment patterns change over time, we can expect that their respective autospectra will display a broadening of the dominant period. If this does occur without affecting the nature of the interaction between the two species, then coherencies will also appear to be significant over a wider range of periods. This process should be investigated further through detailed assessment of the age structure of the fishery and recruitment dynamics of walleye and perch.

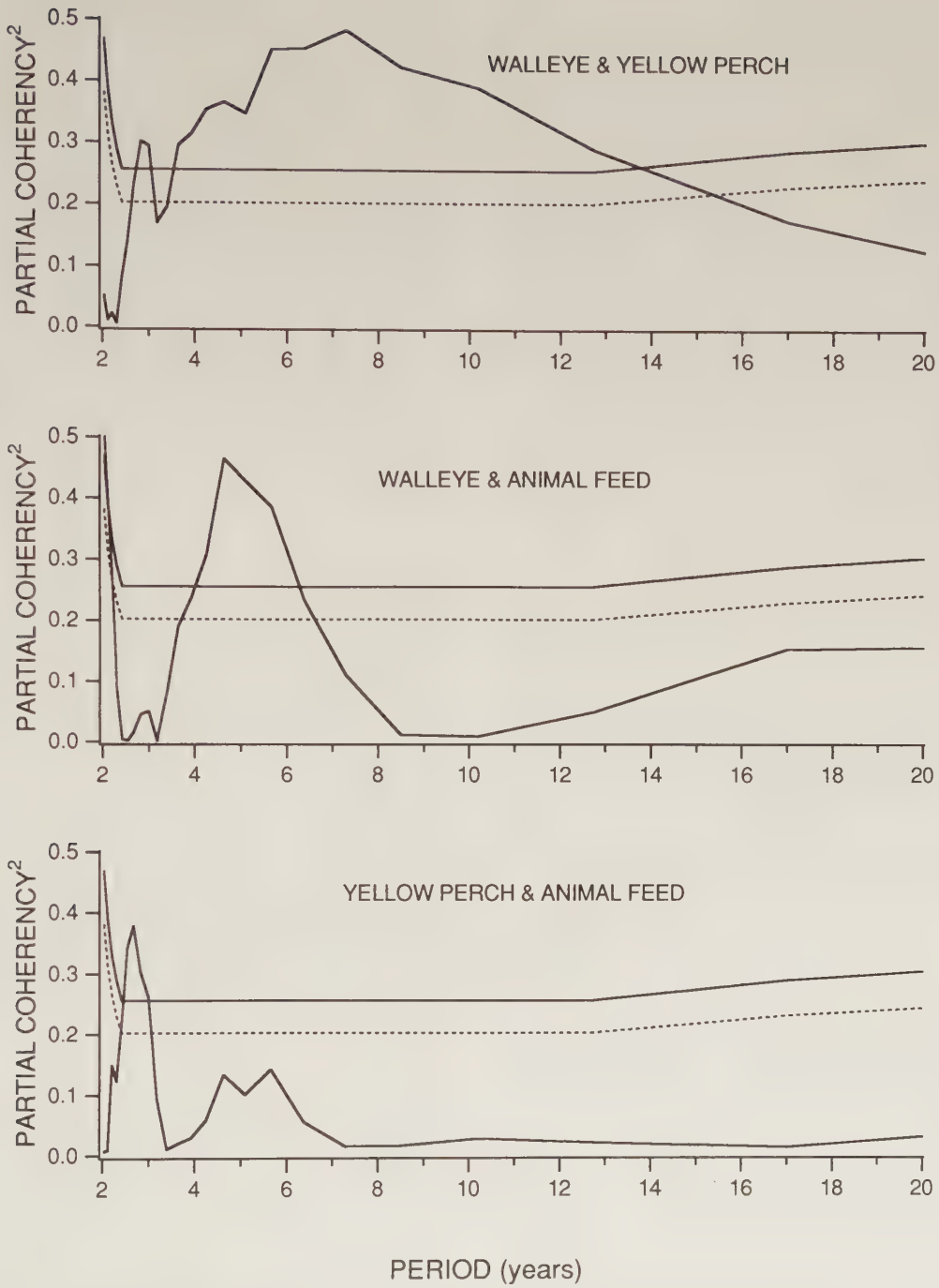

FIG. 4. Squared partial coherencies for all three pairs of transformed and detrended walleye, yellow perch, and animal feed CPUE. The solid and broken horizontal lines represent significant coherency at p values of 5 and 10%, respectively.

Recruitment and Life History of Freshwater Drum

In the Red Lakes, dynamics of drum are characterized by the complete absence of many year classes punctuated by the episodic establishment of a strong year class. Because the species displays slow growth, strong year classes recruit gradually to the fishery. Commercial CPUE will therefore not index recruitment dynamics over relatively narrow time frames. This pattern gives rise to a dominant period in the autospectrum which is considerably larger than that of walleye and yellow perch. Therefore, spectral analysis and the calculation of coherency for drum provide little or no indication of species relationships.

The dynamics of freshwater drum in the Red Lakes may in part be explained by considering its geographic distribution, which extends southward to Guatemala (Scott and Crossman 1973). The Red Lakes are close to the northern limit of drum distribution. Therefore, thermal constraints may explain the observed stunting of this species. This further suggests that ovarian development and spawning may be sporadic from year to year. This phenomenon has been observed over the range of other fish species (Mann et al. 1984; Mills 1988). The persistence of drum in the Red Lakes appears to represent an example of bet-hedging theory (Mann and Mills 1979), which suggests

TABLE 2. Age and size composition of freshwater drum in the 1989 commercial fish harvest. From the total harvest, a sample of 1392 fish were measured for length, and a further subsample of these fish stratified by length was obtained for age determination. Two fish of the 1988 year class were sampled in the fishery with mean total lengths and weights of 185 mm and 66 g, respectively. Additionally there were five fish between the sizes of 219 and 250 mm which were sampled for length but were not aged. Collectively, the year classes from 1986 through 1988 represent only a trace percentage of the total harvest.

Year class	Percent of total harvest	Mean total length (mm) (SD, *n*)	Mean weight (g) (SD, *n*)
1969	2.7	346 (14.0, 7)	498 (72.4, 7)
1970	60.1	321 (24.2, 61)	381 (93.6, 60)
1971	0.5	340 (*n* = 1)	457 (*n* = 1)
1983	33.8	280 (13.9, 38)	254 (41.2, 38)
1984	1.9	281 (3.5, 2)	265 (23.3, 2)
1985	0.5	275 (*n* = 1)	224 (*n* = 1)

TABLE 3. Age and size composition of an initial sample of freshwater drum larger than 2 kg. Fish of this size were not subsampled in the fishery due to their rare occurrence and were therefore obtained by direct purchase from fishers in 1990.

Year class	Mean total length (mm) (SD)	Mean weight (kg) (SD)
1947 (*n* = 2)	621 (29.7)	3.75 (0.354)
1955 (*n* = 10)	588 (44.5)	2.9 (0.469)

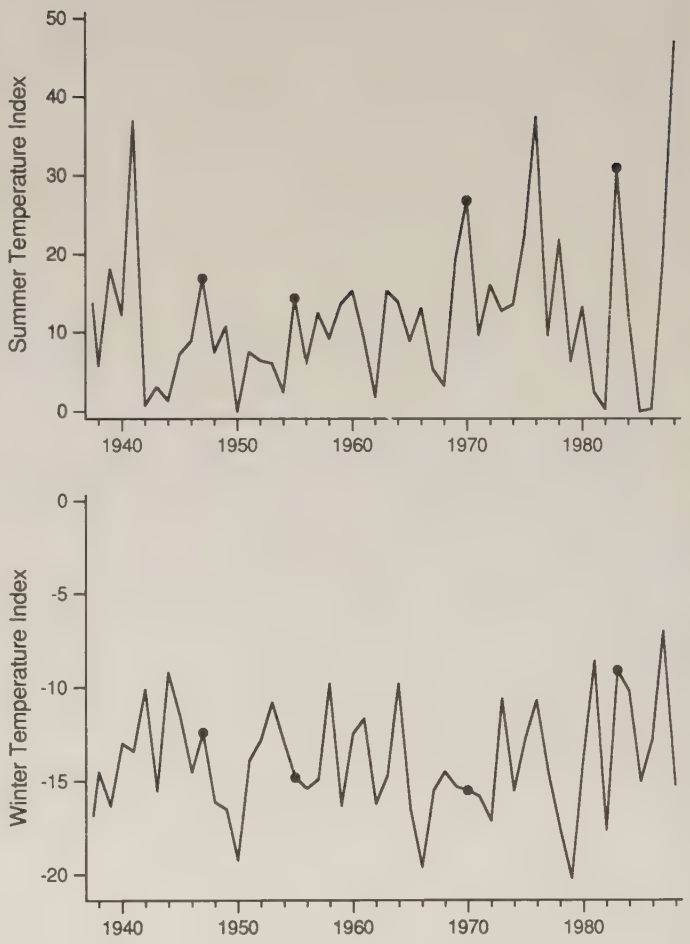

FIG. 5. Summer and winter temperature indices derived from air temperature data from Crookston, MN, 1938–88. The summer temperature index is cumulative degree-days above a base of 25°C summed from June through September. The winter index is the average temperature for January and February. The solid dots on each temperature index indicate the extant year classes of drum.

that iteroparity and increased longevity are life history traits to counter reproductive uncertainty. Bet-hedging theory therefore is consistent with our findings pertaining to the age distribution of freshwater drum. Reproductive uncertainty may be due to either lack of spawning attempts by adults or variable prereproductive survival. Additional work on the Red Lakes should determine the cause of reproductive uncertainty in freshwater drum. This may be accomplished by determining the relative abundance of early life stages from year to year. For example, relatively consistent densities of eggs and larvae followed by variable numbers of fall young-of-the-year and/or yearlings will suggest that reproductive effort is uniform, while prereproductive survival varies annually.

Species Interactions with Freshwater Drum

If prereproductive survival varies, successful recruitment of drum may depend on the coincidence of several factors, including proper temperature patterns (e.g. a warm growing season) and a relaxed predatory gauntlet. In percid communities such as the Red Lakes, the walleye is considered a top piscivore with opportunistic food habits (Ryder and Kerr 1978). Diet will therefore be determined by relative abundance and size distribution of the prey assemblage, and preferences by walleye. In other systems, it appears that walleye may not prey heavily on drum. This may be due to a stronger preference for other prey items (Knight et al. 1984); also, juvenile drum may grow quickly through the size range susceptible to walleye predation (Piegel 1963). However, freshwater drum have buoyant eggs and early larvae which are vigorous but relatively immobile (Davis 1959), and therefore may be highly susceptible to pelagic planktivores. This suggests that a predator–prey relationship may exist between perch and drum. Preliminary Red Lakes data collected in 1988 indicate some spatial overlap between perch postlarvae and drum eggs and larvae. In late June, perch ranged from 15 to 20 mm and drum larvae were approximately 4 mm (A. McClure, Department of Fisheries and Wildlife, University of Minnesota, St. Paul, MN 55108, pers. comm.). Larval fish predators need not be large, and estimations of capture success for a 16-mm perch on a 4-mm drum are about 39% based on functional relationships presented by Miller et al. (1988). Other potential predators of drum eggs and larvae in the Red Lakes include the spottail shiner (Smith and Kramer 1964) and the emerald shiner (*Notropis atherinoides*). Predation on eggs and larval drum will be temporally (i.e. size dependent) and spatially complex (Houde 1989).

Coherency between drum and both walleye and yellow perch at periods shorter than approximately 10 yr is difficult to interpret. Coherency between two species may arise from several processes, including biotic interactions such as competition or

predation and also from dynamic characteristics of the fishing process. Fishery dynamics may encompass such factors as fisher behavior, market supply and demand, and gear effects such as saturation by one particular species inhibiting capture efficiency for another species. As an illustration of potential fishery dynamics, we calculated the Pearson correlation coefficient for the contemporaneous (i.e. time lag of zero) detrended series of walleye and animal feed. This yielded a significant ($p < 0.05$) value of -0.282, suggesting that in years of high walleye CPUE, animal feed CPUE is low. One tenable explanation arises when we consider the market value of these species and practical considerations on the part of the fishers. Walleye is the primary economic species, with fishers in recent years obtaining between $2.20 and $3.30 (U.S.) per kilogram for this species. In contrast, fishers receive six cents per kilogram for freshwater drum (i.e. animal feed). Therefore, in years of high walleye CPUE (i.e. good fishing cussess), fishers have little incentive to transport drum to the processing facility. Alternatively, drum may be turned away at the facility if large walleye harvests place considerable demand on processing and/or storage capacity.

Additional research on the Red Lakes fish community should place initial priority on description of energetics and reproductive biology of drum. We are conducting further, detailed evaluation of recruitment and growth patterns of freshwater drum. This includes additional analysis of large drum purchased from fishers and spectral analysis of growth and climate.

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Morphology of the Toxin-Producing Diatom *Nitzschia pungens* Grunow forma *multiseries* Hasle

Daniel J. MacPhee[1] and Louis A. Hanic

Department of Biology, University of Prince Edward Island, University Avenue, Charlottetown, P.E.I. C1A 4P3, Canada

Dianne L. Friesen

Department of Pathology and Microbiology, University of Prince Edward Island, University Avenue, Charlottetown, P.E.I. C1A 4P3, Canada

and David E. Sims

Department of Anatomy and Physiology, University of Prince Edward Island, University Avenue, Charlottetown, P.E.I. C1A 4P3, Canada

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The toxin-producing diatom *Nitzschia pungens* Grunow forma *multiseries* Hasle from three toxic blooms in two Prince Edward Island estuaries, spanning 1987–89, was studied using light and electron microscopy. Cell ultrastructure of *N. pungens* is, in general, similar to that of other species of *Nitzschia* and other diatoms. Important features include prominent peripheral, polarized nucleoli (numbering one or two) and imperforate poroids, present on inner valves and girdle bands. Cell division is usually synchronous for all cells in a filament with respect to polarity and time. Postdivisional elongation of the filament appears to involve a "slide-by" process whereby sibling cells slide by each other along their opposed valve faces and then stop, becoming fused by their overlapping tips. The raphe is probably involved in this, as well as in filament and cell motility. Observations of particle motion along the cell raphe suggest the existence of a motility apparatus such as microcilia which would facilitate locomotion, intercellular coordination, and the postdivisional slide-by process. No bacteria or other organisms were observed associated with field samples of toxic *N. pungens* f. *multiseries*. This supports a view that domoic acid production is autonomous.

La présente étude porte sur la diatomée *Nitzschia pungens* Grunow forma *multiseries* Hasle productrice de toxine. Des échantillons en ont été recueillis de 1987 à 1989 lors de trois poussées phytoplanctoniques dans deux estuaires de l'Île-du-Prince-Édouard, puis étudiés par microscopie optique et électronique. En général, l'ultrastructure cellulaire de *N. pungens* ressemble à celle d'autres espèces du genre et d'autres espèces de diatomées. Les principales structures comprennent des nucléoles proéminentes périphériques et polarisées (au nombre de un ou deux) et des pores imperforés présents sur l'hypovalve et la ceinture. La division cellulaire est généralement asynchrone chez toutes les cellules d'un filament pour ce qui est de la polarité et du moment. L'élongation du filament après la division semble comporter un processus de "glissade" par lequel les cellules filles, suivant un plan parallèle aux deux valves, s'éloignent l'une de l'autre, puis s'arrêtent lorsqu'elles se fusionnent à leurs pôles superposés. Le raphé joue probablement un rôle dans ce processus, tout comme dans le mouvement du filament et de la cellule. Des observations du déplacement de particules le long du raphé portent à croire à la présence d'un appareil locomoteur comme des micro-cils qui faciliteraient le mouvement, la coordination entre les cellules et le processus de glissade après la division. Aucune bactérie ni autres organismes ont été observés dans les échantillons de *N. pungens* f. *multiseries* toxiques recueillis sur le terrain. Cette observation étaye la notion que la production d'acide domoïque est autonome.

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Nitzschia pungens Grunow forma *multiseries* Hasle is a pennate, chain-forming, motile, planktonic marine diatom widely distributed in coastal waters (Hasle 1965). It is the suspected source of toxin in three successive years of mussel poisoning in Prince Edward Island (P.E.I.), Canada (Bates et al. 1989). The first and most serious episode occurred in the autumn of 1987, when three deaths and over 100 illnesses followed consumption of toxic mussels (Perl et al. 1990). Domoic acid (DA), a potent neurotoxin isolated from mussels, was determined to be the cause of the poisonings (Wright et al. 1989). Sustained, almost unialgal blooms of *N. pungens* occurred at several sites during all three episodes. Plankton hauls of blooms tested positive for DA, leading to the conclusion that *N. pungens* f. *multiseries* was the source of DA. Supporting evidence came when cultures of f. *multiseries* were found to produce DA (Subba Rao et al. 1988, 1990; Bates et al. 1989, 1991). However, none of the cultures studied was axenic, allowing the possibility that DA production in *N. pungens* is due to an infection (i.e. bacterial, fungal, viral).

Infective agents including viruses (Hoffman 1978) and eubacteria (Paerl and Kellar 1978; Nozaki et al. 1989) have

[1]Author to whom reprint requests should be directed. Current address: Department of Zoology, University of Western Ontario, 1151 Richmond St., London, Ont. N6A 5B7, Canada.

been recorded in algae. Cyanobacteria have been reported in several diatoms (Drum and Pankratz 1965; Heinbokel 1986) as ecto- or endosymbionts. However, none of these has been associated with toxin production.

The present study provides additional light microscopical and ultrastructural information on *N. pungens* f. *multiseries* morphology. No evidence was found for the presence of an infective agent in this species.

Materials and Methods

Plankton samples were taken with a 10- to 20-μm-mesh plankton net from the Cardigan River and the Brudenell River (Table 1). Sample 1 was fixed in 3% glutaraldehyde in filtered seawater and stored at 5°C for 10 mo prior to osmication and embedding. Sample 2 was transported to the laboratory at ambient seawater temperature (0°C) where it was concentrated with a 10-μm-mesh screen and fixed for 2 h at 5°C in an excess of freshly prepared 3% glutaraldehyde in filtered seawater. Sample 2 was then filtered, rinsed with seawater, and postfixed for 12 h in 1% osmium tetroxide in seawater. The cells were concentrated with a 0.4-μm filter and placed in 3% agar cylinders with an internal cavity (approximately 2–3 mm in diameter, 3 mm long). The cylinders were capped with agar, heat sealed, dehydrated in ethanol, cleared in propylene oxide, and infiltrated with an epon–araldite mixture. They were cut into pieces, placed in moulds, and polymerized for 12 h at 65–75°C.

Samples 3 and 4 were fixed in 2.5% glutaraldehyde in 0.05 M sodium cacodylate buffer, pH 7.4, in 21 ppt saline water with or without 0.1% ruthenium red. After 12 mo of storage at 5°C, they were concentrated in agar blocks and processed as with Sample 2.

Thin sections were cut with a diamond knife, mounted on bare grids or formvar-carbon coated grids, and poststained with uranyl acetate and lead citrate. Serial sections were viewed for studying certain structures (e.g. pyrenoid membranes, raphe canal, joining of silica frustule and girdle bands).

For scanning electron microscopy (SEM), cells were fixed in 3% glutaraldehyde in seawater or cacodylate buffer as above. Samples were centrifuged lightly, washed in distilled water, and rinsed in ethanol. Drop-samples were placed on SEM stubs, air-dried, and then sputter coated with gold. For frustule morphology and species determination, cells were acid cleaned either by the method of Hasle and Fryxell (1970) or a modification where, following the addition of sulfuric acid, the solution was heated to boiling. In either case, three passes through the procedures were used to assure removal of all organics. (The remaining silica frustules, when placed on a slide and treated with 1% hydrofluoric (HF) acid for a few minutes, completely dissolved, indicating that organics, including a cuticle if present, were removed.) Drops of the diatom solution were placed on SEM stubs, air-dried, and coated with gold. Thirty cells from each collection were taken at random for photography and measured for key features (number of costae and poroids per unit length).

Thirty cells from whole mounts were examined by light microscopy (LM) for cell length, width, and general structure. Whole mounts of glutaraldehyde and osmium fixed samples were stained with toluidine blue for nucleolar number or were treated with 1% HF acid for several minutes for removal of the silica wall component for studying the nature of cell-tip overlap.

Cell division and movement were studied from living material of all four samples and cultures of clone NPARL (a DA-producing clone of *N. pungens* f. *multiseries* obtained from Dr. S. Bates, Department of Fisheries and Oceans, Gulf Fisheries Centre, Moncton, N.B.). Cultures were grown at 15–20°C in 27 ppt salinity Instant Ocean (Aquarium Systems, 8141 Tyler Blvd., Mentor, OH 44060, USA), made with P.E.I. well water supplemented with Fritz (f_2) Algae Food (Fritz Chemical Co., Dallas, TX 75217, USA).

Results and Discussion

Field Data

Two forms of *N. pungens* were encountered in this study: f. *multiseries* was present in all samples and f. *pungens* was present in Samples 3 and 4 (Table 1). F. *pungens* has also been reported from the Cardigan River in 1989 (Smith et al. 1990), where it was found to predominate during the early bloom period. Cultures of f. *pungens* taken from the September 20, 1989, sample and from the Cardigan River (I. Novaczek, P.E.I. Food Tech. Centre, 550 University Ave, Charlottetown, P.E.I., C1A 4P3, pers. comm., and Smith et al. 1990, respectively) were found to produce no DA. The two forms are morphologically very similar. The distinguishing characteristic is that f. *pungens* has large poroids which occur in two rows; in f. *multiseries* the poroids are small and occur in three to five rows (Hasle 1965). The poroids of both forms in our samples were not resolvable by LM. They were readily seen by SEM, although only on the inside of the valves.

The occurrence of f. *pungens* in eastern P.E.I. has two important implications. As pointed out by Smith et al. (1990), using *Nitzschia* as an indicator species for DA levels is only valid where the species forms and their frequencies are known. This means that identifications must be supported by SEM data. Earlier distributional studies of *Nitzschia* in eastern P.E.I. were based mainly on LM data (i.e. Hanic and Cormier 1989; Bates et al. 1989). It is probable that f. *pungens* was occasionally present, which limits interpretation of the data unless archived samples can be reexamined by SEM.

Nitzschia pungens f. *multiseries* was almost the sole phytoplankter in Sample 2. At the time of sampling, the bloom

TABLE 1. *Nitzschia* sampling sites and cell and DA concentrations.

Sample No.	Sample site	Date (d/mo/yr)	Bloom period	Concentration (cells·L^{-1})	Abundance (% by number)[a]	% f. *pungens*	% f. *multiseries*	DA in plankton (μg dry weight^{-1})
1	Cardigan	10/12/87	End	2×10^7	>95	~0	~100	—[b]
2	Cardigan	19/12/88	End	1×10^5	>95	~0	~100	673
3	Brudenell	20/09/89	Start	—	?	81	19	0
4	Brudenell	02/11/89	End	—	>95	8	92	1060

[a]Abundance of *N. pungens* f. multiseries plus f. *pungens* in the phytoplankton, given as a percentage of total cells (all species).

[b]Sample was taken 1 d later but at the same site as in Sample 1. Sample tested positive for DA by mouse bioassay (E. Todd, Health and Welfare Canada, Ottawa, Ont., Canada).

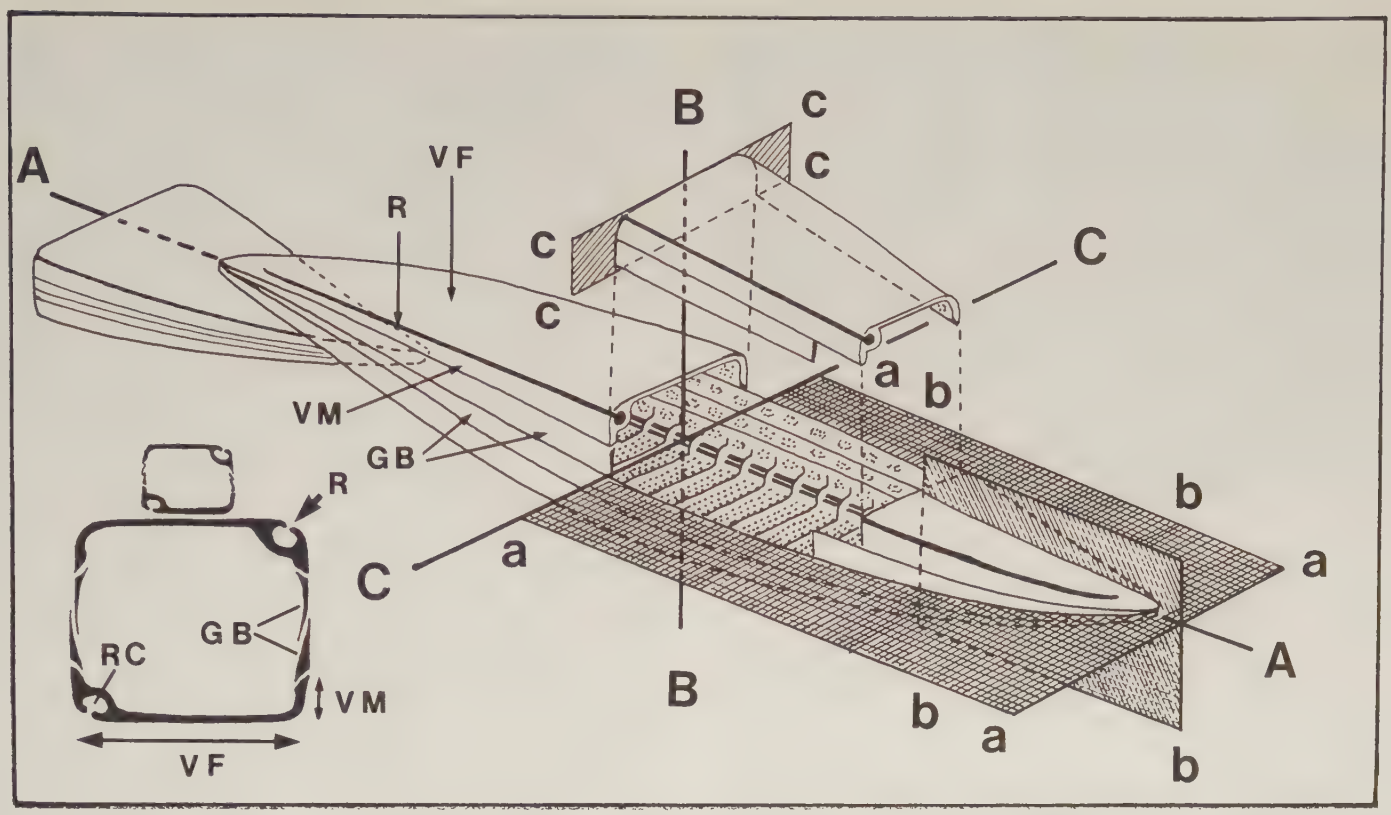

FIG. 1. Silica frustule of *N. pungens* f. *multiseries* shown diagramatically, with cell length much reduced. Reference axes and planes: A, apical axis; B, principle or pervalvar axis; C, transapical axis; a, valvar plane; b, apical plane; c, transapical plane. Raphe (R) on edge of upper valve face (VF) and valve mantle (VM). Section of upper valve removed to show raphe canal (RC), costae, three to five rows of poroids in the intercostal areas and rectangular depressions, containing several poroids on the inner side of girdle bands (GB). Diagram in left-hand corner shows cross-section of two overlapping cells with the raphes in the normal diagonally opposite position.

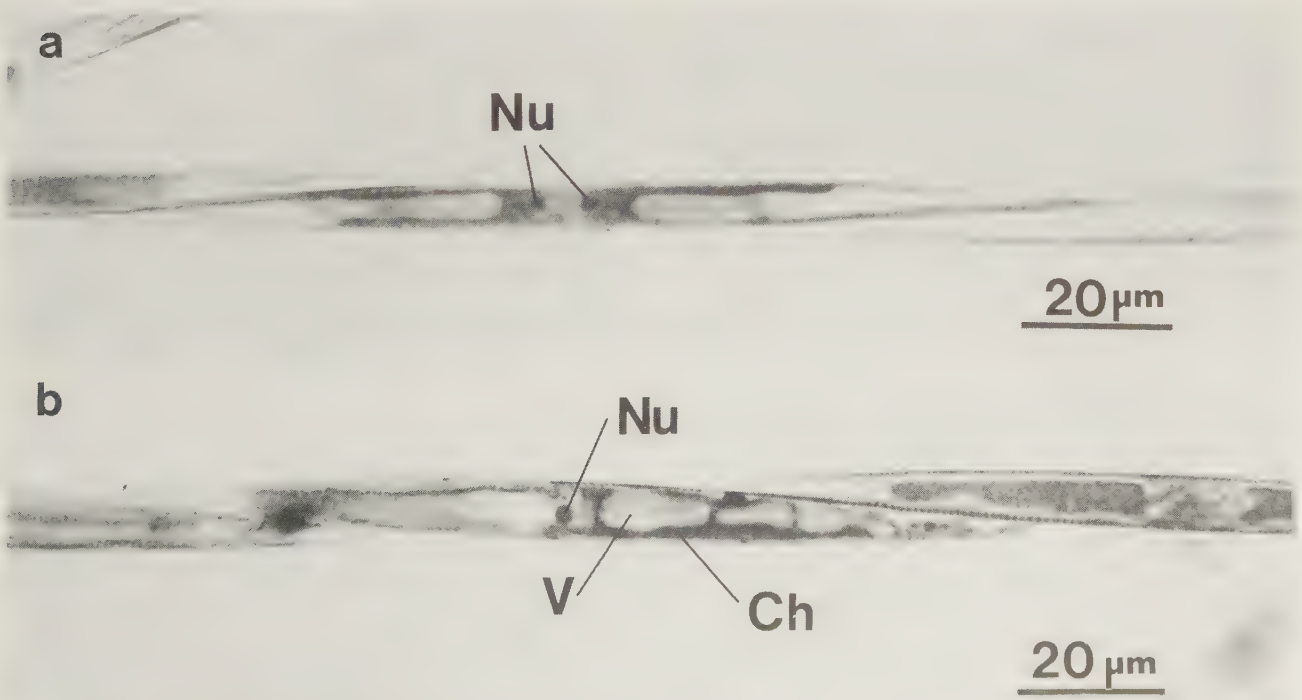

FIG. 2. Filaments from Sample 4 in optical apical section fixed for TEM stained 10 mo later with 1% toluidine blue. Girdle view, central cell showing chloroplasts (Ch), vacuoles (V), and nuclear area, with two nucleoli (Nu) in Fig. 2a and one nucleolus in Fig. 2b.

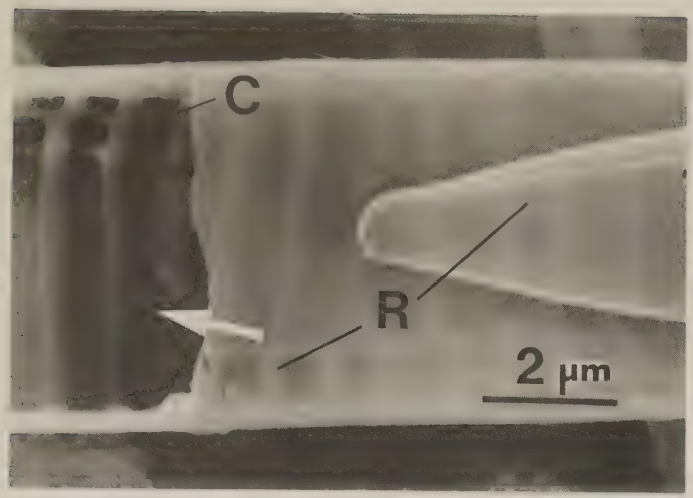

FIG. 3. Sample 2, sulfuric acid cleaned preparation showing overlapping cells, lower cell missing part of upper valve, inner part of lower cell showing costae (C), and intercostal areas (arrow), with well-defined inner openings to the poroids. Note upper cell (right) is attached to lower cell, indicating cementing material was not removed by acid treatment. Note also that raphe of upper valve of this cell is on diagonally opposite side of cell (normal position), compared with that of cell below. In bottom cell, raphes are opposite in mirror image (the abnormal) position.

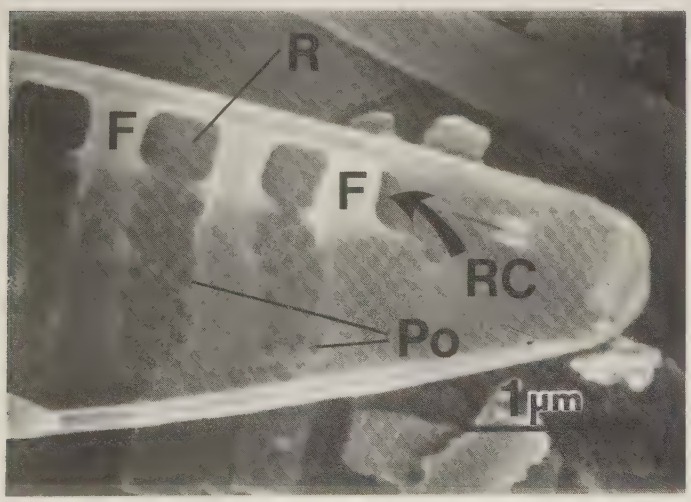

FIG. 4. Sample 2, sulfuric acid cleaned cells showing tip of inner valve with poroids (Po), raphe (R) with terminus, and fibulae (F) bridging raphe canal (RC).

had declined in concentration to 0.1×10^6 cells·L^{-1} from a maximum level of 1.2×10^6 cells·L^{-1} reached earlier on November 14, 1988. Sample 2 yielded a DA content of 673 μg·g dry weight^{-1} which is significant, although considerably lower than the peak of 11 900 μg·g^{-1} recorded from a plankton sample from the Cardigan River, January 1, 1988 (Bates et al. 1989).

All four samples were viewed alive by LM within an hour of collection. Sample 2, which was extensively used for our ultrastructural studies, was critically examined by both bright-field and phase-contrast microscopy. In all samples, *Nitzschia* occurred in long chains and appeared healthy and actively dividing. Filaments exhibited motility when placed on a glass slide and illuminated. Outside surfaces appeared clean and free of epiphytic bacteria or other organisms.

Morphology

The morphology of *N. pungens* f. *multiseries* is presented in diagrammatic form (Fig. 1), by LM (Fig. 2), by SEM (Fig. 3 and 4), and by transmission electron microscopy (TEM) (Fig. 5–10). Details of the silica frustule of *Nitzschia* (shown in Fig. 1) are barely discernable by LM (Fig. 2). Externally the valvar surface, mantle, and girdle bands appear smooth under the SEM (Fig. 3) with the underlying costae and poroids appearing at times as shadows. The raphe lies along one edge of the valve face as a very fine slit (approximately 20 nm wide) (Fig. 1, 3, and 4) with edges projecting pucker-fashion into the much larger raphe canal (1400 nm in diameter; Fig. 4). The raphes of overlapping valves lie in the same plane but do not make contact (Fig. 1 and 5). Normally, the two raphes of one cell lie diagonal to each other (Fig. 5, upper cell), but occasionally lie opposite each other in the manner of *Hantzschia* (Fig. 5, lower cell). Internally, most conspicuous are the costae with their thick fibulae bridging the raphe canal and the poroids of the intercostal areas (Fig. 4) and girdle bands (Fig. 1). In both forms of *Nitzschia*, we found the poroids to be imperforate before and after sulfuric acid treatment; that is they did not penetrate to the outside of the silica frustule (Fig. 1 and 6). We were unable to determine if the remaining silica dome was perforated with smaller pores as is the case in *Navicula pelliculosa* (Riemann et al. 1966). Special attention was given to the area of cell-tip overlap, looking for the possible presence of perforate poroids (punctae) such as might be required to provide cytoplasmic connection between adjacent cells. None was found.

Girdle bands appear to encircle the cell without a break (Fig. 1). Bands are deepest in the midregion of the cell and taper gradually to almost nothing at both poles. On the inside, they have a single row of regularly spaced rectangular depressions (Fig. 1 and 5) lined with several conspicuous poroids. During cell enlargement, additional girdle bands are formed which appear to be incorporated in the sibling cells following division. The valve mantle is shallow (Fig. 1 and 5), allowing for flexing during cell enlargement. The edge of the mantle is bluntly and convexly rounded. The abutting edge of the girdle band is also blunt but concave, fitting into the mantle in the manner of a hinged flap (Fig. 5). This construction allows slight outward flexing of the girdle band, which enables expansion during division and maintenance of cell width.

The intact cell with cytoplasmic contents as seen under LM in girdle view (Fig. 2) is slightly sigmoid, curving at the overlapping cell tips. By LM, conspicuous cell structures are chloroplasts (one or two in each pole), large elongate vacuoles (one in each pole), and a central, clear, nuclear area. The nucleolus was not visible in the living cell. Toluidine blue applied after fixation in glutaraldehyde and osmium tetroxide was found to be effective in staining nucleoli, even in material stored for up to 16 mo at 5°C in the dark (Fig. 2). Staining revealed the nucleus to contain either one or two nucleoli, the number of nucleoli being constant for all cells of any one filament. The nucleoli always appeared to be peripheral within the nucleus and polarized.

Ultrastructurally the cell contents of *N. pungens* f. *multiseries* (from Sample 2 only) in general appear similar to those of other *Nitzschia* species (Drum 1963; Pickett-Heaps 1983) and other pennate diatoms (e.g. Drum and Pankratz 1964). The nucleolus is prominent (Fig. 7). This feature, together with it

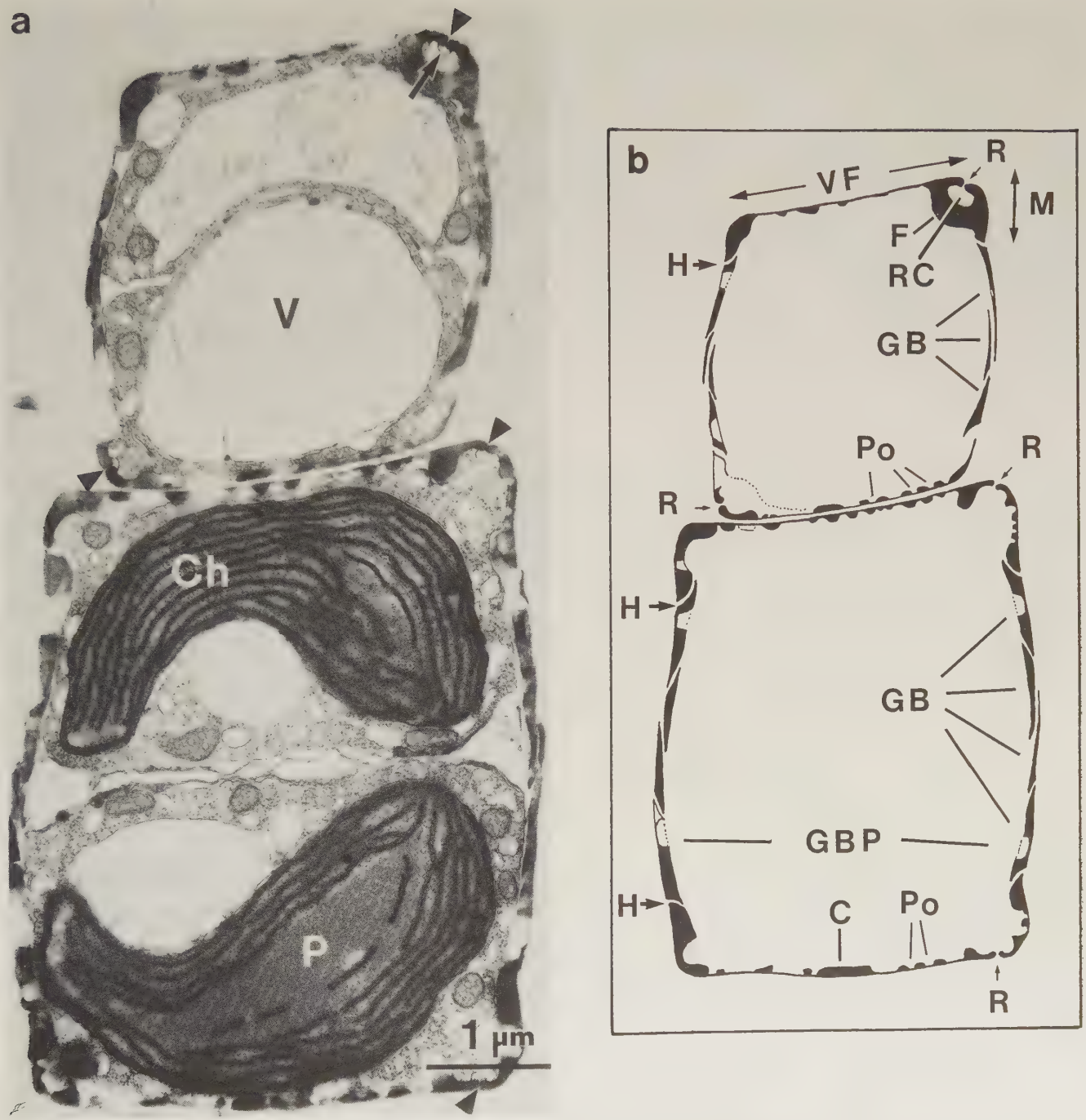

FIG. 5. (a) TEM and (b) diagram of paratransapical plane section from Sample 2 through overlapping tips of two cells in synchronous division. (a) Upper cell sectioned closer to tip showing large vacuoles (V) and cytoplasm extending into raphe canal but drawn back from raphe to form cylinder-like structure (arrows). Lower cell section containing chloroplast (Ch) in each of two sibling cells. Pyrenoid (P) enclosed in membrane and transected by ribbon- or tube-like thylakoids. Raphes (arrowheads) in normal diagonal position in upper cell and in abnormal adjacent (*Hantzschia* species) position in lower cell. Note absence of silica connections between adjacent valve faces. (b) Interpretation of silica wall component from three serial sections (including section in Fig. 5a). Note hinge-like joint (H) between edges of valve and adjacent girdle band allowing for swelling and maintenance of valve width. Valve face (VF), mantle (M), raphe (R), raphe canal (RC), fibulae (F), girdle bands (GB), girdle band poroid areas (GBP), costae (C), and poroids (Po).

being peripheral and polarized, could indicate that the cell is in active protein synthesis (Ghadially 1988).

Golgi stacks (Fig. 7) are prominent and perinuclear, indicating secretory activity. They are often more concentrated adjacent to nucleoli (Fig. 7) and contain several cisternae from which arise numerous vesicles.

The chloroplasts (Fig. 7–10) are long flattened structures appressed to the cell wall. Centrally they press up to, and often extend into, the nucleus (Fig. 7). Distally they sometimes extend just to the point of cell overlap but may also extend into the cell tip. The chloroplast contains thylakoids stacked in layers of three or four (Fig. 8) with the outer thylakoid enveloping

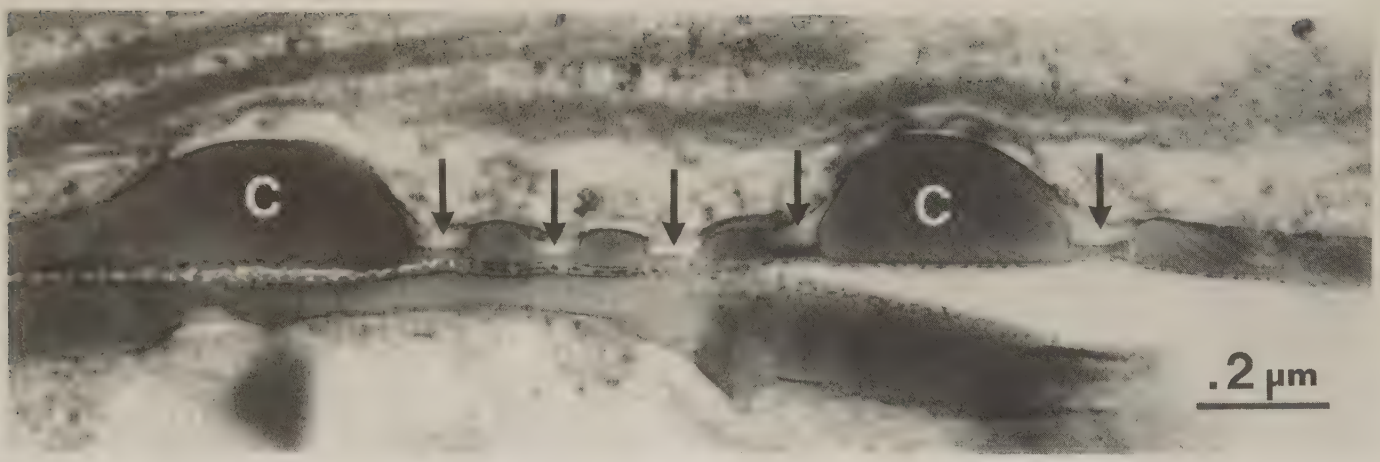

FIG. 6. Sample 4, paratransapical section through overlapping valves showing silica component of costae (C) and imperforate poroids (arrows) of intercostal areas.

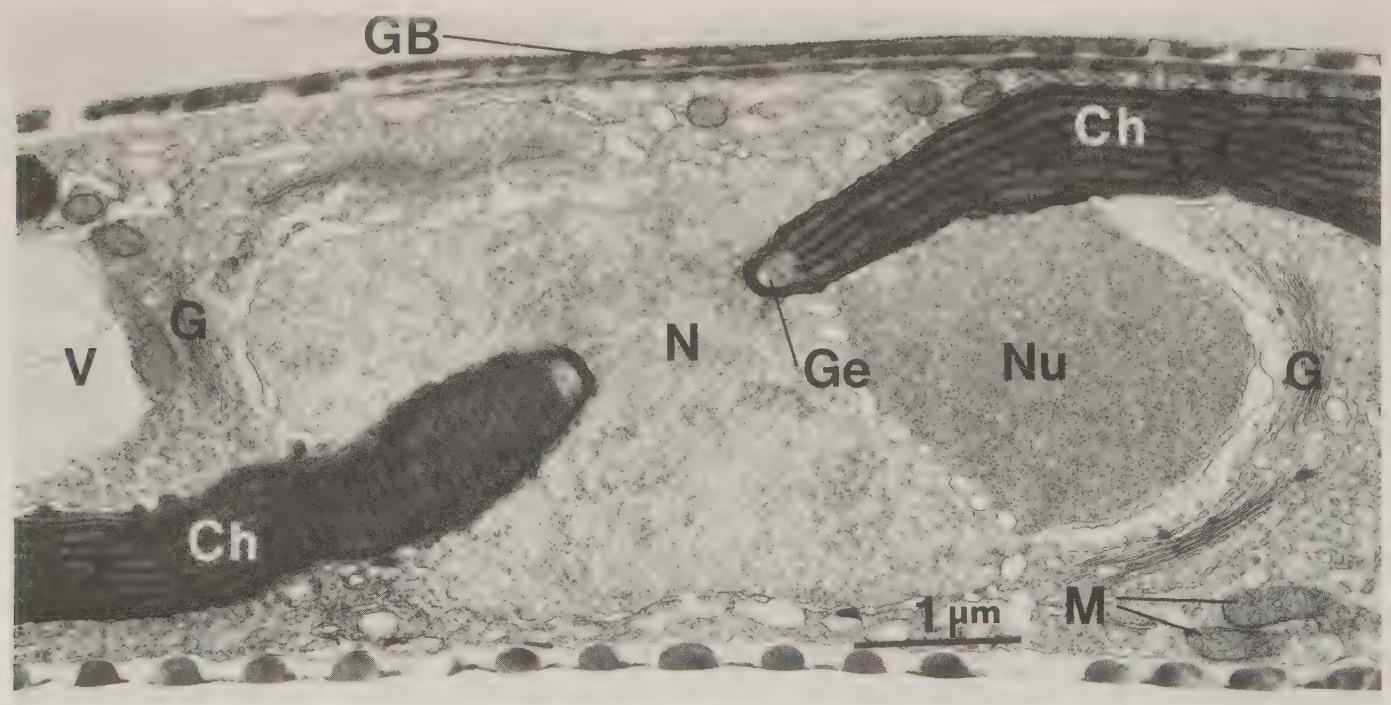

FIG. 7. Paravalvar plane section through overlapping girdle bands (GB). Nuclear area showing oval nucleus (N), polar nucleolus (Nu), perinuclear Golgi (G) with associated vesicles, chloroplasts (Ch) pressing into the nucleus, genophore (Ge), vacuole (V), and mitochondria (M).

the whole chloroplast. A clear area (Fig. 7) is found at one edge of the chloroplast, probably the site of the genophore. Internally the chloroplast contains one or more long membrane bound pyrenoids (Fig. 5, 9, and 10) which are transected by one to three narrow ribbon- or tube-like thylakoids.

The vacuole (Fig. 2, 5, and 7) is large and often vesiculated. It lies central to the chloroplast, extending from the nuclear area to the cell tip.

Mitochondria are numerous and occur throughout the cell (Fig. 5, 7, and 10). They contain tubular cristae and are about 0.3 µm in diameter and up to 20 µm long (Fig. 10).

The cytoplasm extends into the spaces between costae (Fig. 9), appressing the underside of the intercostal areas and poroids of the valves. The cytoplasm also extends into the raphe canal sometimes forming a cylinder-like structure (Fig. 5). The raphe in *N. pungens* in cross-section (described earlier as resembling two inward puckering lips) differs from nonchain-forming, motile *Nitzschia* species. In *N. palea*, one lip projects into the other, tongue and groove fashion (Drum 1963). In *N. sigmoides* the lips pucker outward markedly (Pickett-Heaps 1983).

Motility

Suspended cells and filaments of *N. pungens* do not exhibit motility. Cells and filaments exhibit motility, however, when placed on a glass slide with a coverslip and illuminated. The difference is that under a coverslip, cells and filaments are forced into contact with each other, and with glass surfaces. A single filament placed in a hanging drop also does not exhibit motility while in suspension or following contact with the surface water film. Likewise, two or more filaments do not exhibit

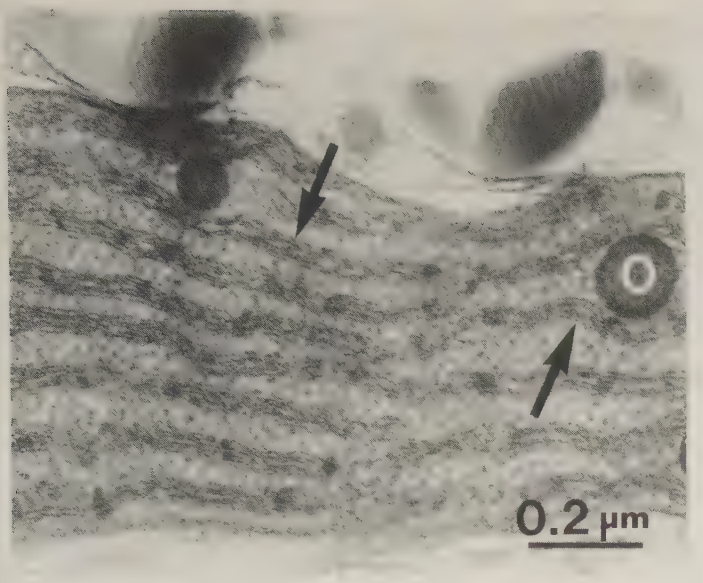

FIG. 8. Chloroplasts showing thylakoids containing stacks of three or four lamellae (arrows) and osmiophillic granules (O).

motility while separated. However, as the filaments fall to the bottom of the drop and make contact, motility occurs. In this situation, one filament is serving as a substrate for the other. While there may be more than one purpose for motility, one function could be for dispersal in the water column, to maximize access to light and nutrients.

Motility is expressed by filaments (or individual cells) gliding along their long axis for a period, stopping, and then reversing direction. The gliding speed is rapid (25 $\mu m \cdot s^{-1}$), and uniform, with motion lasting in any one direction from seconds to minutes. The constant speed suggests coordination of movement between cells. However, considering that we observed no connecting pores between valves of adjacent cells and that the raphes of the valve of one cell do not make contact with the raphes of the valve of the adjoining cell, it would seem that coordination by direct cytoplasmic contact can be ruled out. A mechanism involving indirect cytoplasmic contact, however, may be involved. The following observations bear on these two points.

When fine particles of pumice are added to a hanging-drop preparation of *N. pungens*, one or more particles attach to the cells along the raphes. They move along the cell(s) at the same speed exhibited by filaments moving on glass. Particles on adjacent cells move in the same or in opposite directions and have independent starting or stopping times. Particles on the same cell move in the same direction. Elongate pointed fragments of pumice, when affixed by the pointed end, trail by the other end, swinging about the sharp point (of contact) during directional changes. Single particles (especially large ones) and clusters of particles passed from cell to cell and often moved over the length of several cells. Particles were also seen to drop off when reaching the tip of the last cell of the filament. These observations suggest that motile, sticky cytoplasmic elements project from the raphe and have the ability to hold onto a substrate and, through some unknown manner, (perhaps ciliary-like action?) move the cell along. Besides providing for motility, microcilia could also provide a sensory mechanism for intercellular coordination. Movement of one cell within a filament could entrain movement in the other cells, resulting in all cells (of the filament) moving in the same direction at the same speed as that of one cell. However, we found no ultrastructural evidence of motile microciliary elements.

Cell Division

The raphe and associated motility apparatus may also be involved in filament elongation following cell division. In field and culture samples, all cells in a filament divide synchro-

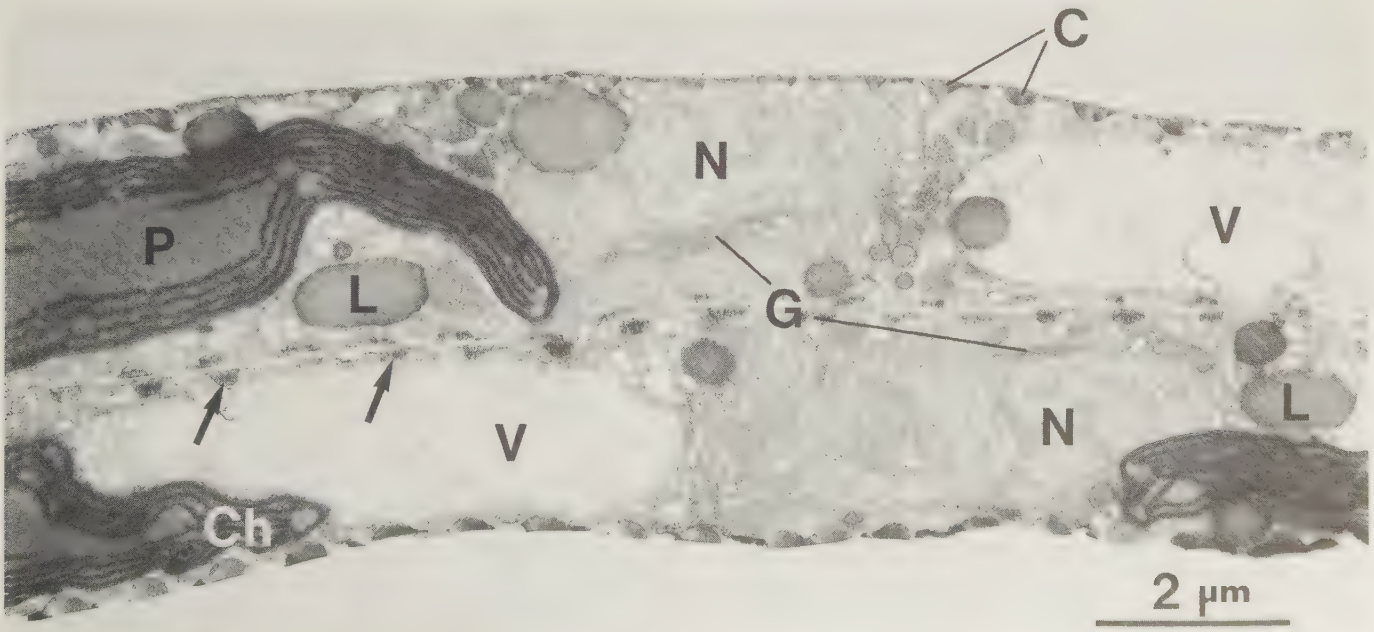

FIG. 9. Paraapical plane section of dividing cell through nuclear area showing nuclei (N), vesiculated vacuole (V), scattered lipid droplets (L), Golgi (G), and chloroplasts (Ch) with pyrenoid (P). Silica walls seen in apical plane showing costae (C) and costal initials (arrows).

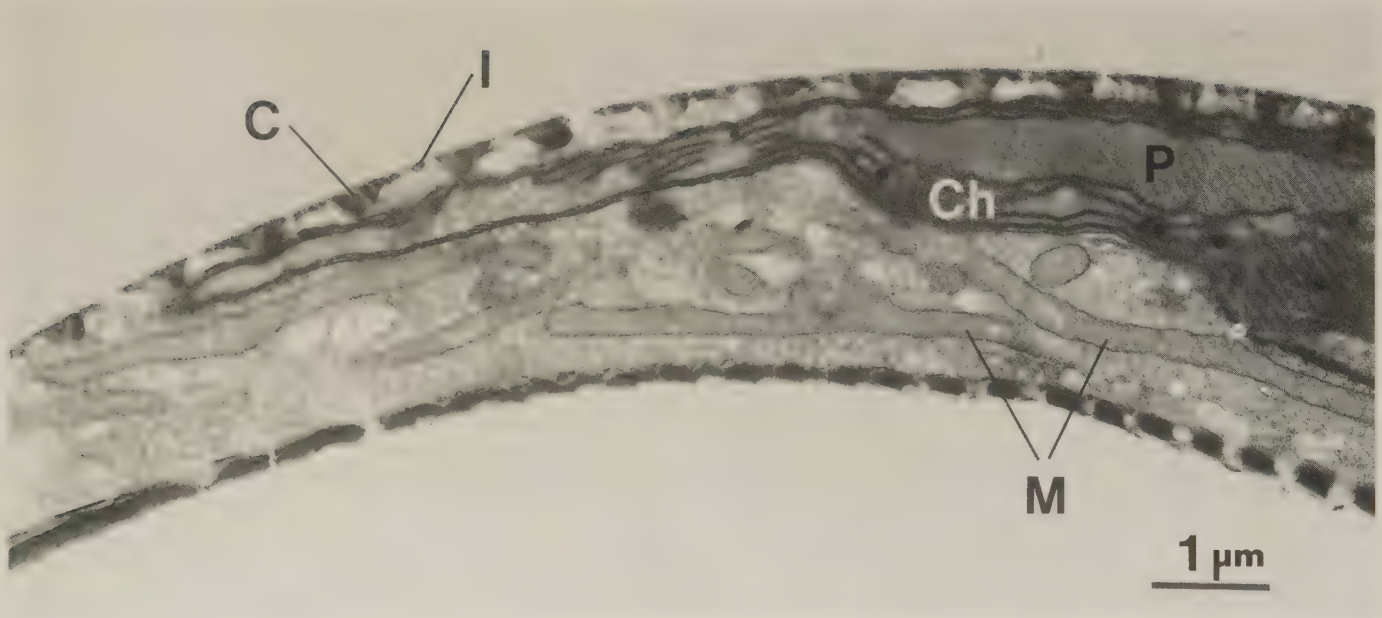

FIG. 10. Paraapical plane section of cell showing long mitochondria (M), chloroplast (Ch) with pyrenoid (P), costae (C), intercostal area (I), and poroids.

nously, or nearly so. Furthermore, separation of sibling cells is synchronously polarized. During division, the first new valve components laid down are the costal initials (Fig. 9), followed by the intercostal areas. Following this, we infer that the two sibling cells slide past each other along the plane of their newly formed and opposed valve faces, at the same time staying in line with the axis of the filament. The actual sliding was not seen. However, filaments in various stages of the "slide-by" process were seen. The post divisional slide-by process is probably the same as that involved in cell motility. When the valve surfaces of the sibling cells are one half to three quarters exposed, the remaining opposed tips become rigidly fused. The joint does not dissolve in HF acid and resists considerable manipulation (i.e. the cells remain in filaments). We were not able to determine if silica deposition was completed before or after the slide-by and fusion process. If deposition was incomplete, the newly formed valves would be more flexible and allow closer valve contact. There would also be an avenue through the cell wall (other than through the raphe) for extrusion of cementing material.

A comparable slide-by process also operates in filament extension and motility in the chain-forming pennate diatom *Bacillaria paradoxa* (Drum and Pankratz 1966). As in *N. pungens*, sibling cells of *B. paradoxa* slide along their valve faces following division. However, in *Bacillaria*, this is not followed by fusion of the tips. Rather, the cells remain free to move independently and continuously along their faces, held together by the opposed raphes which fit into each other like two interlocking rails (Drum and Pankratz 1966). When *Bacillaria* filaments are fully extended, they bear a very close resemblance to filaments of *N. pungens*. It should be pointed out that should *B. paradoxa* be present in a fixed plankton sample, some filaments would likely be in the fully extended state. These, if viewed by LM alone, could be falsely identified as *N. pungens*.

Endo- or Exosymbionts?

In approximately 100 cells from Sample 2 examined by TEM, no evidence was seen of external or internal bacteria, fungi, or encapsulated viruses. We cannot rule out the complete absence of bacteria or viruses because adherent agents may have been washed off during sample preparation, but we are confident that large concentrations (as would presumably be needed to affect cell physiology) would have been encountered. These results lend support to a view that DA production in *N. pungens* f. *multiseries* is autonomous.

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Depuration of Domoic Acid from Live Blue Mussels (*Mytilus edulis*)

I. Novaczek,[1] M. S. Madhyastha, and R. F. Ablett

Prince Edward Island Food Technology Centre, Box 2000, Charlottetown, P.E.I. C1A 7N8, Canada

A. Donald

Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, 550 University Ave., Charlottetown, P.E.I. C1A 4P3, Canada

G. Johnson

Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, 550 University Ave., Charlottetown, P.E.I. C1A 4P3, Canada

and M. S. Nijjar and D. E. Sims

Department of Anatomy and Physiology, Atlantic Veterinary College, University of Prince Edward Island, 550 University Ave., Charlottetown, P.E.I. C1A 4P3, Canada

Novaczek, I., M. S. Madhyastha, R. F. Ablett, A. Donald, G. Johnson, M. S. Nijjar, and D. E. Sims. 1992. Depuration of domoic acid from live blue mussels (*Mytilus edulis*). Can. J. Fish. Aquat. Sci. 49: 312–318.

Industrial depuration may provide a means of removing domoic acid toxin from blue mussels (*Mytilus edulis*). Mussels containing up to 50 μg domoic acid·g^{-1} were transported from a Prince Edward Island estuary into controlled laboratory conditions to test the effects of temperature, salinity, mussel size, and feeding upon depuration. Fifty percent of toxin was eliminated within 24 h. After 72 h, mussels were either clean or contained, on average, only residual levels of toxin (<5 μg·g^{-1}), regardless of conditions. Exponential depuration curves were fitted to the domoic acid concentration data. To evaluate differences in rate of depuration under various conditions, statistical comparisons were made between slopes of the clearance curves. Rates of depuration were faster in small (45–55 mm) than in large mussels (60–70 mm) and more rapid at 11 than at 6°C. There was no significant difference in depuration rate at 18‰ salinity as opposed to 28‰ or in starved versus fed mussels. Because of their relatively large digestive glands, meats of small mussels contained more toxin per unit weight than meats of large mussels. The bulk of domoic acid appeared to reside in the gut lumen. However, the presence of small amounts of domoic acid in intracellular compartments cannot be ruled out.

La dépuration industrielle peut être un moyen d'éliminer la toxine qu'est l'acide domoïque chez la moule bleue (*Mytilus edulis*). Des moules contenant jusqu'à 50 μg d'acide domoïque·g^{-1} recueillies dans un estuaire de l'Île-du-Prince-Édouard ont été transportées dans un laboratoire pour y étudier les incidences de la température, de la salinité, de la taille des individus et de l'alimentation sur la dépuration. On a ainsi déterminé que 50 % de la concentration de toxine était éliminée en deçà de 24 h. Après 72 h, les moules étaient soit libres de toxine ou n'en contenaient, en moyenne, que des concentrations résiduelles (< 5 μg·g^{-1}), indépendamment des conditions. Des courbes exponentielles de dépuration ont été lissées en fonction des données sur les concentrations d'acide domoïque. Afin d'évaluer les différences entre les taux de dépuration dans diverses conditions, on a effectué des comparaisons statistiques entre les pentes des courbes d'élimination. Les taux de dépuration étaient plus élevés chez les petites moules (45–55 mm) que chez les gros individus (60–70 mm); de plus, la dépuration était plus rapide à 11 qu'à 6°C. Il n'y avait toutefois aucune différence significative du taux de dépuration à une salinité de 18 et de 28‰ ou entre les moules affamées et les moules nourries. À cause de leur grosse glande digestive, les petites moules contenaient plus de toxine par unité de poids que les grosses moules. La plus grande partie de l'acide domoïque semblait être accumulée dans la lumière du tube digestif. Toutefois, la présence de petites quantités d'acide domoïque dans les compartiments intracellulaires ne peut être ignorée.

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Domoic acid occurring as a natural contaminant of cultivated blue mussels (*Mytilus edulis*) acts as a neuroexcitatory amino acid that has proven lethal for elderly human consumers (Todd 1989). This toxin was first found in mussels harvested from eastern Prince Edward Island (P.E.I.) in the autumn of 1987 and has recurred, to a lesser extent, in each subsequent year. In 1990, only trace levels of domoic acid were found in cultivated mussels. Domoic acid enters mussels when they ingest the diatom *Nitzschia pungens* f. *multiseries* (Bates et al. 1989), which may bloom in localized areas of P.E.I. from late September through December. Virtually all of the toxin found in mussels appears to reside in the digestive gland (Wright et al. 1989).

[1]Author to whom correspondence should be addressed.

The Canadian Federal Department of Fisheries and Oceans prohibits harvesting of shellfish that contain more than 20 μg domoic acid·g^{-1}. To date, mussel processors have been able to continue operations using mussels from uncontaminated estuaries. However, evidence from the phytoplankton monitoring program of the Department of Fisheries and Oceans suggests that the carrier organism has a much broader geographic range than originally thought, so this option may not always be available. The potential for depuration of domoic acid from live mussels was therefore of commercial as well as academic interest.

In the past, depuration has been attempted for decontamination of live shellfish containing microbes (Canzonier 1971), heavy metals (Schulz-Baldes 1974; Riisgard et al. 1987; Lobel and Marshall 1988; Chassard-Bouchard and Galle 1988), algal toxins (Shumway 1990), and other poisons (Fossato and Canzonier 1976; Mattson et al. 1988; Pruell et al. 1986). The success of depuration varies with the type of chemical or microbe, the species of shellfish, the physiological condition of the shellfish, and physical factors such as temperature (Cunningham and Tripp 1975; Canzonier 1988; Shumway 1990). In some cases, agents such as UV light or ozone have been applied in an attempt to reduce contaminant levels; in other cases, manipulations of environmental factors such as salinity and temperature have been tested to determine whether natural depuration processes can be stimulated. Lipophilic contaminants are believed to depurate slowly because they are readily bound in intracellular compartments (Spacie and Hamelink 1985). Being polar and essentially hydrophilic (Wright et al. 1989), domoic acid might be relatively difficult to bioaccumulate and easy to excrete, making it a good candidate for removal through depuration techniques.

In the present study, a series of experiments was designed to test the effects of temperature, salinity, mussel size, and feeding on depuration of naturally toxic mussels held under controlled laboratory conditions. Temperatures and salinities tested were within the range found in P.E.I. estuaries or salt wells employed as water sources by mussel processors. It was hypothesized that domoic acid in contaminated mussels could reside in two general compartments: extracellular spaces, such as the lumens of the stomach and digestive gland, and intracellular spaces. If domoic acid does not cross gastrointestinal membranes to enter intracellular spaces, a single elimination curve (Spacie and Hamelink 1985) should model the depuration data well. Intracellular domoic acid would presumably clear from the system more slowly. Provided this component was large enough to be distinguished against variability in gut content, the elimination curve for mussels having domoic acid in this second compartment should be biphasic.

Materials and Methods

Mussel Depuration

Collections of cultivated mussels, naturally contaminated with domoic acid, were procured on four sampling dates during the period 2 November to 1 December 1989. Each sample was collected from a single mussel cultivation sock suspended from the surface in the Brundenell estuary, P.E.I. Ambient water temperatures ranged from 9°C on 28 October to 2°C on 6 December (J. White, Department of Fisheries and Oceans, Charlottetown, P.E.I., pers. comm.). Mussels were transported in a cooler to holding facilities in the Fish Health Unit, University of Prince Edward Island, where they were manually declumped and then acclimated overnight in air at the planned experimental temperature. Mussels were graded into two size groups, i.e. 60–70 mm long (large) or 45–55 mm long (small), with mussels outside of these grades being discarded. Size-graded mussels, in groups of nine, were aligned in Vexar No. H52 net socks (Dupont Canada Inc., Whitby, Ont.) so that the exhalent siphons pointed downwards. Each Vexar sock was randomly assigned a number. The No. 1 sock in each treatment series was taken as a sample at time 0, this being the time at which the remaining socks were immersed in freshly prepared artificial seawater (Instant Ocean, Aquarium Systems, Mentor, OH) of predetermined salinity and temperature. At intervals of 6–24 h (depending upon the experiment, see below), two socks were sampled from each treatment following the order of the previously assigned numbers.

Depuration units comprised 22-L plastic containers maintained at a constant temperature (6 or 11°C) by partial immersion in a 1000-L bath of running water at 5 or 10°C. All units were stocked with the same number of socks, to a maximum of nine socks per unit. Clean seawater of 18 or 28‰ salinity was delivered at a constant rate, without recirculation. Overflowing seawater from the depuration units was allowed to fall into the cooling bath.

Ammonia concentration was monitored using Seatest ammonia kits (Aquarium Systems, Mentor, OH) at 6-h intervals during the day. Koch rings having an established bacterial flora were placed in each depuration unit together with the mussels to control ammonia. The flow rate of incoming seawater was maintained such that the ammonia in every unit in a given trial did not exceed 0.4 μg·mL^{-1} at any time. Flow rates of 4 L·h^{-1} as well as partial water exchanges each evening were required in experiments at 11°C where 18‰ salinity was employed. Lower flow rates of 2 L·h^{-1} were used when all units were kept in 28‰ or at 6°C.

To minimize reingestion of faeces, a plastic grid with a 1-cm mesh was placed on the bottom of each container to trap solids. Each unit was continuously aerated using a 2 × 2 × 5 cm, large-pore airstone strapped to a central pipe 10 cm above the bottom. The mussel socks were suspended through holes in a plastic lid. The lids, together with suspended mussels, airstone, and water and food supply lines, were transferred to clean depuration units at 24-h intervals so that the animals were completely removed from contact with their faeces and dissolved excreta.

When the treatment was continuous feeding, food was mixed into each container at a concentration of 5.5 mg·L^{-1} and additional ration was delivered simultaneously and continuously to all treated units using a metered peristaltic pump. Spray-dried Toruteen yeast (U.F.L. Food Products Inc., Montreal, Que.) mixed in seawater was used as a food source. This food was selected for its convenience and economy so that, if effective, it could be realistically recommended for industrial application. The yeast had a particle size range of 1–5 μm. The rate of flow of food was reduced after the removal of each sample so that the water in each unit was slightly colored but never turbid, and there was minimal accumulation of food on the bottom. On average, during feeding trials the ration delivered for each mussel was 4 mg·h^{-1}.

Each sock of experimental mussels was divided into 3 subsamples of three animals each. Thus, each sample of two socks provided six data points. The three drained meats were weighed. Digestive glands (including the gut lumen content, portions of

intestine enveloped by the glands, and adjacent portions of kidney) were dissected out, weighed, and homogenized using a Polytron homogenizer.

Estimation of Domoic Acid Concentration

Extraction and quantification of domoic acid were performed according to the boiling water method of Quilliam et al. (1989). Extracts were analyzed on a Gilson HPLC system consisting of a model 302 pump, a 100-μL loop injector, a Supelcosil LC-18PAH column (15 cm × 4.6 mm I.D., 5 μm), and a variable wavelength UV detector set to 242 nm wavelength and 0.01 sensitivity. The isocratic mobile phase was 9% acetonitrile in water acidified with 0.1% trifluoroacetic acid. Parallel extractions were performed on a reference mussel homogenate (MUS-1) and using a domoic acid analytical standard (DACS), both supplied by the National Research Council of Canada, Halifax, N.S. Quantification was accomplished by comparing the areas of peaks from unknowns with those of standard solutions prepared from the DACS. Using MUS-1, 100% recovery of domoic acid was accomplished routinely.

Concentrations of domoic acid in mussel extracts were calculated in terms of micrograms of domoic acid per gram in mussel digestive glands on a wet weight basis. These values were then extrapolated to micrograms per gram in whole mussel meat, assuming that 100% of the toxin was to be found in the digestive gland as defined above.

Statistical Analysis

The data for decrease in domoate concentration in whole mussel meat over time in each treatment were transformed logarithmically and fitted to curves by linear regression technique (SAS Institute Inc. 1987). Multiple regression analysis of variance was used to determine the statistical significance of differences among depuration curves. The residuals were plotted for each data set. These plots were examined for any consistent pattern that might suggest that a better fit would be obtained using a two-compartment model.

Results

Trial 1. Effect of Salinity at 11°C (Mussels Collected 15 November)

Under conditions of 18 and 28‰ salinity at 11°C, large mussels (60–70 mm in length) with a mean (±SD) domoate concentration of 22.6 ± 12.9 $\mu g \cdot g^{-1}$ lost 50% of their toxin within 24 h and 90% within 48–72 h (Fig. 1). Two replicate units were employed for each salinity treatment. As there were no differences between replicate units, all data were pooled. Domoate concentration reached a residual level of 1 $\mu g \cdot g^{-1}$ within 72 h regardless of salinity.

In this experiment, samples were taken at 0, 6, 12, 24, 36, 48, 72, and 120 h. However, the high degree of variability in domoic acid concentration negated any benefit from sample times less than 24 h apart. There was no difference in slopes of the depuration curves and only a slight improvement in the standard errors of the slopes when the full data set was used for analysis compared with when only 24-h interval data over the 0- to 72-h period were used. The 6-h sampling program was logistically difficult. Because of the poor return for the effort involved, sampling was restricted to 24-h intervals in subsequent trials.

Trial 2. Effects of Mussel Size and Feeding at 11°C (Mussels Collected 20 November)

In this trial, toxic mussels of two size classes (45–55 and 60–70 mm) were tested in parallel. Half of the mussels were fed continuously on yeast while the others were starved. Because the ratio of weight of digestive gland to total meat weight was greater in the smaller mussels (0.24 ± 0.06) than in the larger ones (0.18 ± 0.01), the meats of the small animals were more toxic (21.9 ± 7.8 versus 14.9 ± 2.5 $\mu g \cdot g^{-1}$ in large animals) on a fresh weight basis. Toxin concentration in digestive gland tissue alone was similar in small and large mussels (88.8 ± 18.3 and 82.7 ± 11.3 $\mu g \cdot g^{-1}$, respectively).

In all treatments, the mussels, on average, lost at least 50% of their toxin in the first 24 h and 90% by 48–72 h (Fig. 2). Thus, results were similar to those of trial 1. Rates of depuration were more rapid in the smaller mussels, regardless of feeding.

Trial 3. Effects of Salinity and Feeding at 6°C (Mussels Collected 1 December)

Large mussels (60–70 mm) collected for this trial contained 13.3 ± 3.2 μg domoic $acid \cdot g^{-1}$. Small mussels (45–55 mm) from the same collection demonstrated significantly more ($p < 0.05$) toxin per unit body weight (17.4 ± 1.2 μg $domoate \cdot g^{-1}$).

Large mussels were depurated either at 18 or 28‰ salinity, and in each case, half were continuously fed and the others were starved. After 72 h, some mussels contained less than 1 μg $toxin \cdot g^{-1}$, but others still retained as much as 8.5 μg $domoate \cdot g^{-1}$ (Fig. 3). This was in contrast with the more complete clearance of toxin from mussels at 11°C. The rate of depuration was similar at low and high salinity and was consistently more rapid in the fed mussels compared with starved ones.

Multiple Regression Analysis

According to the analysis of variance (Table 1), both temperature and mussel size had significant effects upon the rate of depuration of domoic acid. Depuration was more rapid in small mussels and at the higher temperature. Salinity was marginally nonsignificant and there was no significant effect attributable to feeding.

Validity of the Single-Compartment Model

In modelling depuration with a single regression line, the working hypothesis was that the toxin resided in a single body compartment. Residuals from the linear regressions of log domoic acid concentration versus time were examined and in most cases these were randomly arranged. There was no consistent pattern suggestive of the retention of any demonstrable amount of domoic acid in a second, slowly clearing compartment.

Discussion

A depuration system for mussels was devised that had flowing seawater of suitable temperature and salinity, was well aerated, and allowed for removal of contaminated faeces. Given such favorable conditions for active filtration, one could hypothesize that mussels might depurate faster at higher temperatures (Bayne et al. 1976), as long as the temperature was

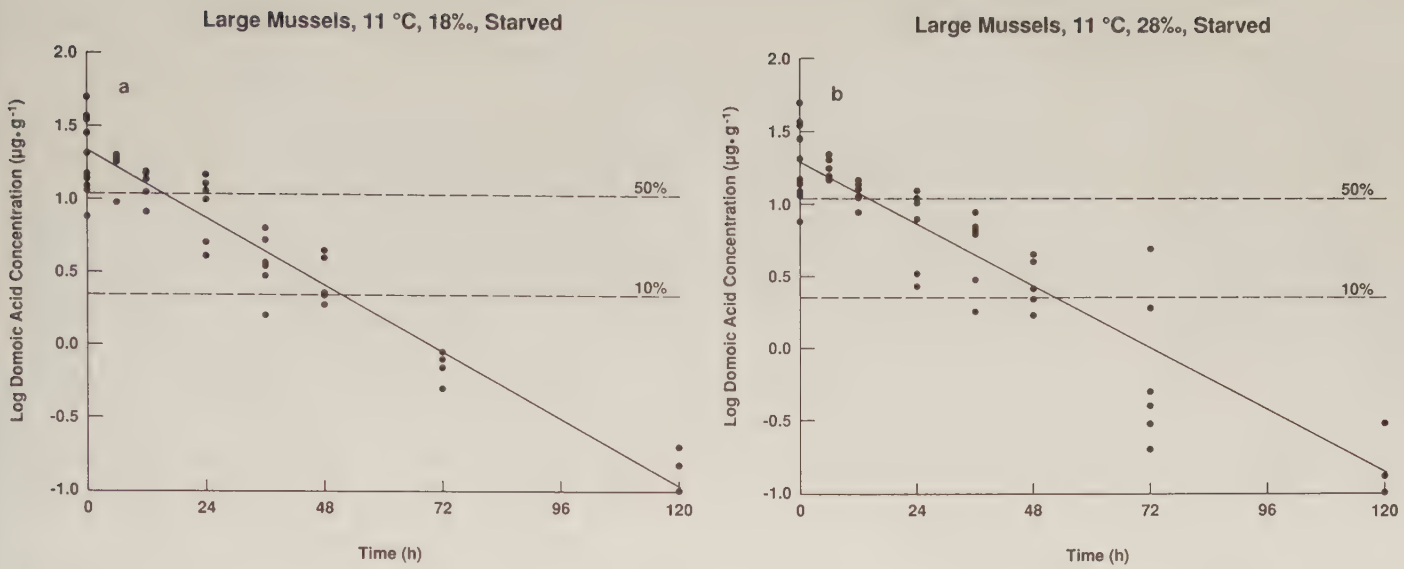

FIG. 1. Trial 1. Domoic acid concentration in whole mussel meat over time at 11°C in large, starved mussels. (a) Salinity 18‰; log DOM = $1.31 - 0.019 \cdot T$; $R^2 = 0.94$. (b) Salinity 28‰; log DOM = $1.30 - 0.018 \cdot T$; $R^2 = 0.88$. Depuration curves fitted by linear regression on log-transformed data. Broken lines indicate 50 and 10% of average initial toxin content.

not so high as to stimulate spawning (Thompson 1984) or cause heat stress. Small mussels might be anticipated to depurate faster than larger ones (Widdows et al. 1979; Tsuchiya 1980; Hawkins et al. 1990; Schulz-Baldes 1974), and continuously fed mussels might depurate faster than starved animals (Hawkins and Bayne 1984) as long as particle loads were not so high as to inhibit filtration (Thompson 1984). If domoic acid occurred as a cytosolic free amino acid in mussel cells, exposure to low salinity might effect a rapid clearance of this compartment (Gilles 1972; Wright et al. 1987).

If naturally occurring domoic acid resided only in the gut cavity of mussels, depuration should proceed in a single rapid stage coincident with gut clearance. Provided that domoic acid acted like a nontoxic food item, gut clearance should be accomplished within 24 h, depending upon temperature (Foster-Smith 1975; Hawkins and Bayne 1984). However, depuration could be prolonged if the toxin interfered with normal digestive and egestive processes. If there was also some uptake of toxin into tissues, two or more stages might be apparent, with the intracellular compartment(s) clearing more slowly.

The curve of log domoate concentration versus time (Fig. 1–3) was modelled reasonably well by a single straight line. The bulk of domoic acid was therefore associated with a rapidly clearing body compartment, probably the gut lumen. As total clearance was not accomplished within 24 h, however, this could indicate that domoic acid has an inhibitory effect on gut evacuation. There was no consistent evidence of the persistence of toxin in any more slowly clearing compartment. Other experimental evidence (Novaczek et al. 1991; Madhyastha et al. 1991) has indicated minor uptake of domoic acid into mussel cells. Clearly, the degree of intracellular incorporation of domoic acid in the naturally toxic mussels used in this study was insignificant compared with the toxin in the gut lumen, as it could not be distinguished against the mussel-to-mussel variability in gut content. However, evidence of long-term storage of residual, and presumably intracellular, toxin (about 1 $\mu g \cdot g^{-1}$ in digestive gland tissue) was noted in 10% of mussels retained in culture for up to 3 mo after exposure to toxic *N. pungens* (I. Novaczek and M. S. Madhyastha, unpubl. data).

Smaller mussels consistently carried more toxin per unit body weight than larger ones and this may be attributed to their relatively heavier digestive glands. In spite of this, smaller mussels cleared their body load of toxin more rapidly than larger mussels. This observation is consistent with previous reports of rapid metabolic rates in young mussels (Schulz-Baldes 1974; Widdows et al. 1979; Tsuchiya 1980; Hawkins et al. 1990).

Low temperature consistently depressed the rate of clearance of domoic acid. This is consistent with evidence that metabolic processes such as filtration rate and assimilation efficiency are temperature dependent (Bayne et al. 1976).

Although depuration tended to be more rapid at the lower salinity, the effect was not statistically significant. If domoic acid does occur as a cytosolic free amino acid, it is either unaffected by salinity change or it is such a small component of total toxin load that fluxes in this compartment cannot be distinguished against the background variability. The effect of feeding was not statistically significant.

On average, mussels were able to purge themselves of toxin to a level of less than 5 $\mu g \cdot g^{-1}$, and in some cases to undetectable levels, within 24–72 h. Extrapolation of the regression between domoate concentration and depuration time to 1 $\mu g \cdot g^{-1}$ indicates that there would be a doubling in depuration time for each 10-fold increase in initial toxin concentration. If this is the case, the extrapolation suggests that no more than 1 wk would be required at temperatures of 6–11°C to cleanse mussels having the highest domoate concentration yet recorded (Bates et al. 1989) of approximately 900 $\mu g \cdot g^{-1}$. However, domoic acid is toxic to other invertebrates (Maeda et al. 1987) and may be significantly toxic to mussels when present over some as yet undetermined threshold level. In this case, the extrapolation would not be valid and depuration times for highly toxic mussels could be longer. On the other hand, larger toxin loads may signify the ingestion of food that contains more concentrated toxin rather than the presence of a larger mass of toxic food. Provided that high concentrations of domoate do not retard defecation or induce unusually large intracellular uptake of toxin, then one would expect mussels to expel a given mass of gut content in the same amount of time regardless of its

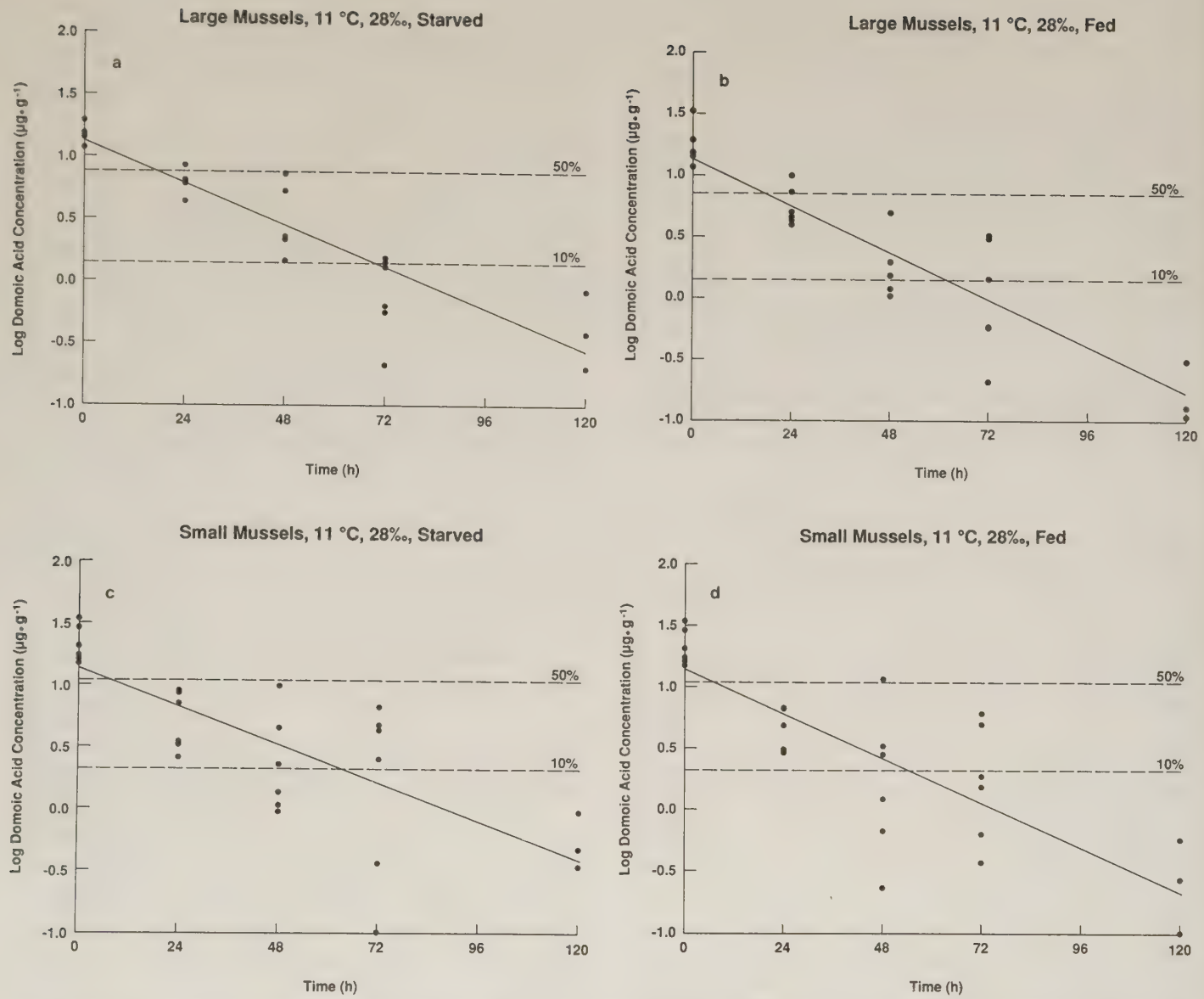

FIG. 2. Trial 2. Domoic acid concentration in whole mussel meat over time at 11°C and 28‰ salinity. (a) Large, starved mussels; log DOM = 1.15 − 0.014·T; R^2 = 0.81. (b) Large, fed mussels; log DOM = 1.13 − 0.016·T; R^2 = 0.85. (c) Small, starved mussels; log DOM = 1.14 − 0.013·T; R^2 = 0.58. (d) Small, fed mussels; log DOM = 1.14 − 0.015·T; R^2 = 0.66. Broken lines as in Fig. 1.

toxicity. Clearly, more work on mussels having a wider range of initial toxin concentrations is required.

In contrast with domoic acid, the algal toxins responsible for paralytic shellfish poisoning (PSP) have not been cleared from mussels in less than 10 d, and depuration may take a number of weeks (Shumway 1990). The rate of loss of PSP toxins appears to change with the season (Prakash et al. 1971) and may be retarded by low temperatures (Madenwald 1985). Attempts to stimulate the depuration of PSP toxins from mussels by manipulating temperature and salinity have yielded little success (Gilfillan et al. 1976; Blogoslawski and Neve 1979). PSP toxins, like domoic acid, are largely restricted to digestive gland and stomach tissue. Unlike domoic acid, PSP toxins are known to be poisonous to mussels and cause adverse effects such as reduced rates of filtration, cell damage in the gut, reduced ciliary activity, and even death (Shumway 1990).

In conclusion, the depuration trials conducted on a laboratory scale indicate the feasibility of removing roughly 90% of the domoic acid from moderately contaminated mussels within a few days. Whether this process will be effective for heavily contaminated mussels or at an industrial scale remains to be tested. The large variability observed in the toxicity of mussels in the field indicates that adequate replication of samples is essential for confident estimation of initial domoic acid concentrations. As long as a reasonable safety margin is employed, guidelines for standard depuration times for mussels of different average initial toxicities should be attainable.

For depuration on an industrial scale, a system of holding tanks for mussels should have the faeces periodically flushed out and/or trapped, and care should be taken not to resuspend faecal material during aeration. Beyond these basic precautions, our study suggests that no special conditions or equipment are needed, as depuration proceeds over a wide range of temperature, salinity, and feeding conditions and in both large and small mussels. Low-salinity salt wells should provide suitable water sources and have the advantage of being higher in temperature than ambient seawater during the latter months (November–December) of the toxic mussel season.

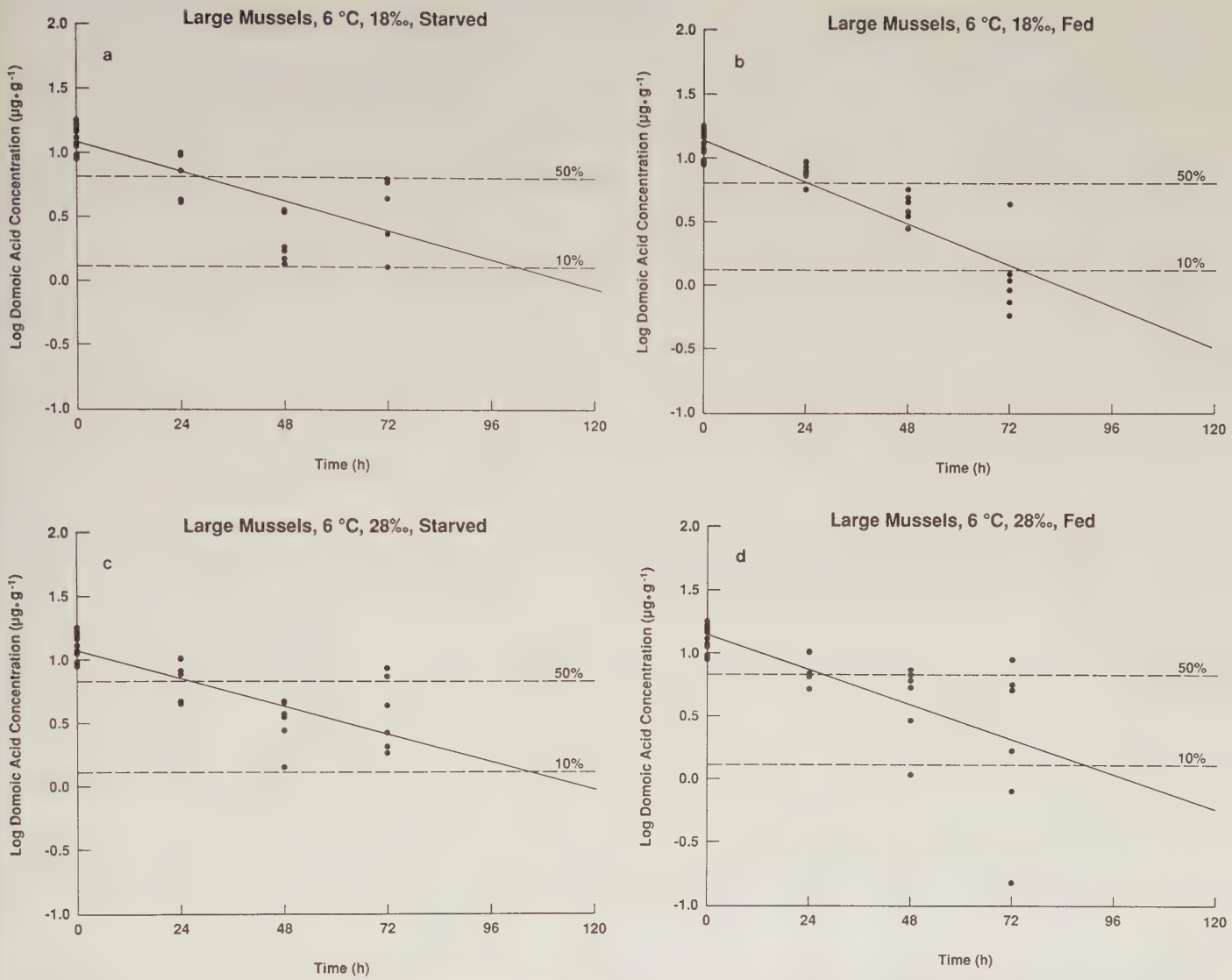

FIG. 3. Trial 3. Domoic acid concentration in whole mussel meat over time at 6°C in large mussels. (a) Salinity 18‰, starved mussels; log DOM = $1.05 - 0.009 \cdot T$; $R^2 = 0.55$. (b) Salinity 18‰, fed mussels; log DOM = $1.16 - 0.014 \cdot T$; $R^2 = 0.84$. (c) Salinity 28‰, starved mussels; log DOM = $1.08 - 0.009 \cdot T$; $R^2 = 0.61$. (d) Salinity 28‰, fed mussels; log DOM = $1.12 - 0.011 \cdot T$; $R^2 = 0.50$. Broken lines as in Fig. 1.

TABLE 1. Results of multiple regression analysis (analysis of variance) indicating the statistical significance of differences in depuration curves (log-transformed data) exhibited by toxic mussels under various conditions of mussel size, temperature, salinity, and feeding. *Significant ($p < 0.05$).

Variable	T statistic	p
Temperature (11 vs. 6°C)	−6.56	0.0001*
Mussel size (small vs. large)	−2.61	0.009*
Salinity (18 vs. 28‰)	1.86	0.06
Food (none vs. continuous)	−1.40	0.16

Finally, until government regulations are in place, depuration cannot proceed on mussels from closed harvesting areas. However, in the interest of both consumers and the industry, individual processors could perform depuration trials on legally harvested mussels containing less than 20 µg domoate·g^{-1}. The simple strategy of holding mussels in running water for 24–48 h before shipping will probably obviate any risk to consumers from these marginally contaminated mussels and thus provide mussel producers and buyers with greater confidence in the marketed product.

Acknowledgements

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Mitochondrial DNA Analysis of Walleye Pollock, *Theragra chalcogramma*, from the Eastern Bering Sea and Shelikof Strait, Gulf of Alaska

Timothy J. Mulligan[1]

Fisheries Research Institute, WH-10, University of Washington, Seattle, WA 98195, USA

and Robert W. Chapman and Bonnie L. Brown

Department of Biology, East Carolina University, Greenville, NC 27858, USA

Mulligan, T. J., R. W. Chapman, and B. L. Brown. 1992. Mitochondrial DNA analysis of walleye pollock, *Theragra chalcogramma*, from the eastern Bering Sea and Shelikof Strait, Gulf of Alaska. Can. J. Fish. Aquat. Sci. 49: 319–326.

Increased fishing effort in the international waters of the Aleutian Basin has focused much interest on defining the stock structure of walleye pollock, *Theragra chalcogramma*, in the Bering Sea. Variation in mitochondrial DNA (mtDNA) was examined via restriction endonuclease digestions in 168 walleye pollock from three areas in the eastern Bering Sea and from Shelikof Strait, Gulf of Alaska. Nine endonucleases produced variant restriction patterns both within and among populations. A total of 50 restriction sites were revealed along the mtDNA molecule. Two dominant genotypes were found in 39 and 21 individuals, respectively. Fifty-one genotypes (78% of the total) were represented by a single specimen. Clustering of genetic distances suggests the existence of several walleye pollock stocks in the Bering Sea and Gulf of Alaska. Genetic divergence among stocks may be related to the prevailing current patterns found in these areas.

Un plus grand effort de pêche dans les eaux internationales du bassin des Aléoutes a mis en relief le besoin de définir la structure du stock de goberge de l'Alaska, *Theragra chalcogramma*, de la mer de Béring. Les auteurs ont étudié la variation de l'ADN mitochondrien après dégradation par endonucléase de restriction chez 168 goberges provenant de trois différentes régions du secteur est de la mer de Béring et du détroit de Shelikof, dans le détroit de l'Alaska. Neuf endonucléases ont donné des cartes de restriction variables au sein d'une population et entre les populations. Au total, 50 sites de coupure ont été identifiés le long de la molécule d'ADN mitochondrien et deux génotypes dominants ont été relevés chez 39 et 21 goberges, respectivement. On a aussi identifié 51 génotypes (soit 78 % du total) chez un seul spécimen. Le groupage des distances génétiques port à croire à l'existence de plusieurs stocks de goberge de l'Alaska dans la mer de Béring et le golfe d'Alaska. L'écart génétique entre les stocks peut être lié aux régimes des courants dominants dans ces régions.

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Walleye pollock, *Theragra chalcogramma*, are a schooling, semidemersal species inhabiting shelf, slope, and basin waters throughout the North Pacific (Hart 1980). Currently, they comprise the most productive single-species fishery in the world with annual landings exceeding 6.75 million tons (FAO 1988) valued at $3.4 billion (Alverson 1990). By weight, they are the dominant fish species in the highly productive Bering Sea ecosystem (Lynde 1984), where a major portion of the commercial catch is taken.

Prior to 1980, most Bering Sea walleye pollock were harvested from within the U.S.S.R. and U.S. 200-mi Exclusive Economic Zones. In the early 1980's, several nations including Japan, Poland, Korea, and China began fishing in the international waters of the Aleutian Basin, known as the "Donut Hole" (see Fig. 1). This fishery takes place mainly during the spawning season with no quotas or management restrictions. Estimated annual catches from the Donut Hole since the mid-1980's are in excess of 1 million tons (Mulligan et al. 1989).

An interesting and unexplained aspect of the population biology of walleye pollock in the Aleutian Basin, including the Donut Hole, is the very low abundance of larval and juvenile fish compared with adjacent slope and shelf areas (Haryu 1980; Mulligan et al. 1989). This, coupled with the substantial multinational fishery for adults, has led to several hypotheses concerning the origin of fish caught in the Donut Hole and has emphasized the importance of defining the stock structure of walleye pollock in the Bering Sea. The hypothesis regarded as most likely by U.S. and Soviet scientists is that walleye pollock from the Donut Hole are derived from adjacent slope or shelf stocks (Dawson 1989; Bulatov and Sobolevsky 1989). If this hypothesis is correct, unregulated fishing in the international waters could severely affect coastal stocks within the U.S. and/or U.S.S.R. fishery conservation zones.

Restriction endonuclease analysis of mitochondrial DNA (mtDNA) has become a powerful tool in the study of animal populations. Recently, it has been used successfully to identify the stock structure of several commercially important anadromous fishes including striped bass, *Morone saxatilis* (Chapman

[1]Present address: Department of Fisheries, Humboldt State University, Arcata, CA 95521, USA.

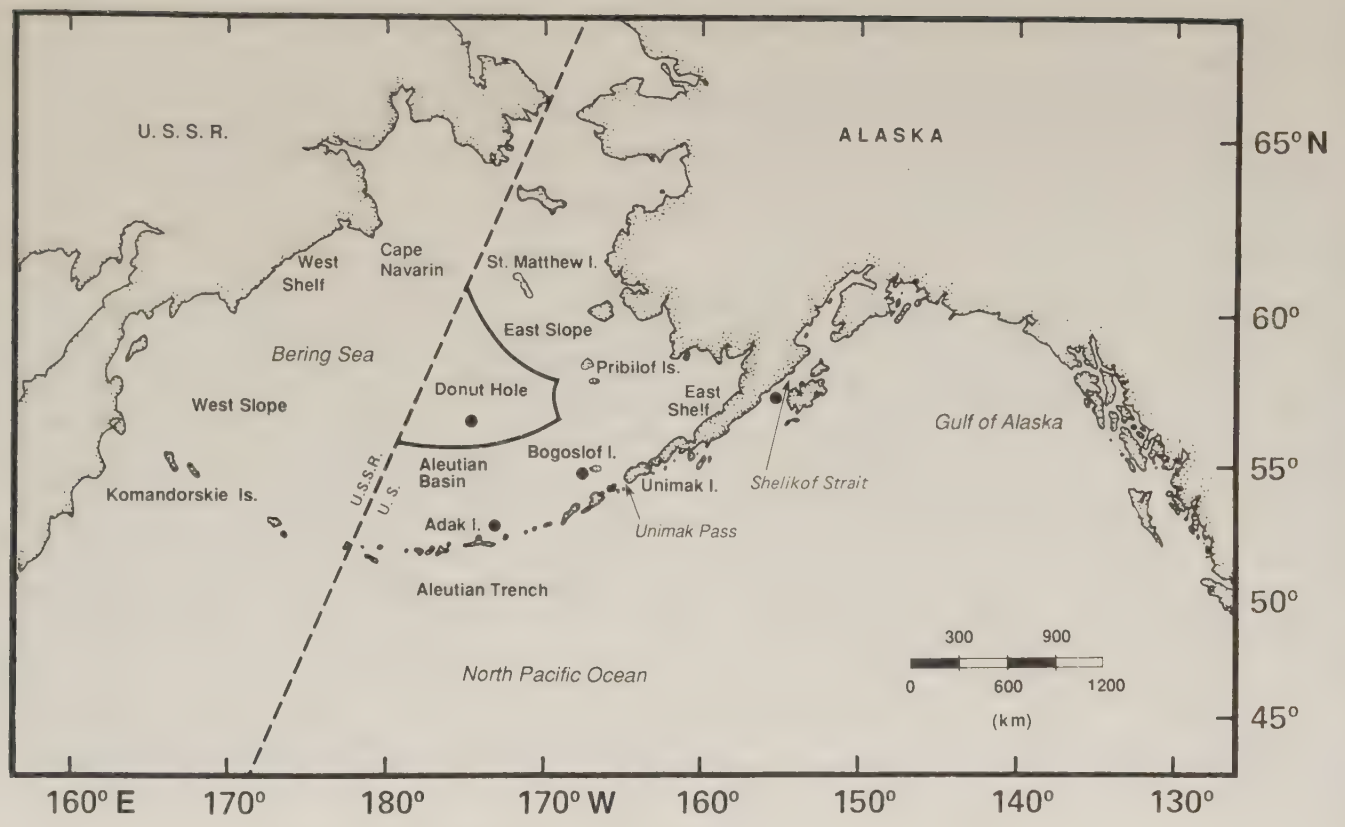

FIG. 1. Bering Sea and Gulf of Alaska. Solid circles designate the four collection sites.

1987, 1989), American shad, *Alosa sapidissima* (Bentzen et al. 1988), and white perch, *Morone americana* (Mulligan and Chapman 1989). Although this approach has been less informative in delineating stocks or marine species (Graves et al. 1984; Kornfield and Bogdanowicz 1987; Graves and Dizon 1989; Smith et al. 1989), the technique is thought to be more sensitive than conventional allozyme studies and thus may expose additional differences among populations (Avise et al. 1986; Kornfield and Bogdanowicz 1987). mtDNA anlaysis is used here to identify walleye pollock stocks in the eastern Bering Sea and Gulf of Alaska. This study has important political and economic implications, as it is the first to examine the genetic relationships between walleye pollock from the Donut Hole and other areas of the Bering Sea.

Materials and Methods

Walleye pollock were collected by 18-m midwater rope trawls during winter and spring 1988. Sampling was conducted in the Bering Sea over the southeastern Aleutian Basin in the vicinity of Bogoslof Island (lat. 53°52′40″N, long. 167°40′19″W), over the southcentral Basin near Adak Island (lat. 52°26′55″N, long. 174°08′32″W), and in the Donut Hole area of the Basin (lat. 56°04′80″N, long. 179°15′53″E). An additional sample was taken in Shelikof Strait, Gulf of Alaska (lat. 57°41′27″N, long. 155°10′70″W) (Fig. 1).

Mature ovaries were removed from each fish and immediately frozen at −80°C onboard the vessel. In the laboratory, approximately 10 g of each ovarian tissue sample was thawed gradually, at 7°C, in 50 mL of STEK buffer (0.25 M sucrose, 50 mM Tris, 10 mM EDTA, 1.5% KCl, pH 7.5). mtDNA was isolated using modifications of the Chapman and Brown (1989) method as described by Mulligan and Chapman (1989). mtDNA samples were digested with the six-base recognition endonucleases *EcoR* I, *Sst* II, *EcoR* V, *Xba* I, *Apa* I, *Hind* III, *Pst* I, *Bgl* I, and *Dra* I. The resulting fragments were separated via horizontal electrophoresis on 0.8% agarose gels using the one-kilobase (kb) ladder (Bethesda Research Labs) as a molecular weight standard. The gels were stained for approximately 30 min with ethidium bromide, destained in deionized water, and photographed according to Chapman and Brown (1989).

Nucleotide sequence divergence between populations was calculated according to Nei and Li (1979). The unweighted pair-group method using arithmetic averages (UPGMA, Sneath and Sokal 1973) was then used to cluster these distances. Statistical confidence in these clusters was tested using heterogeneity chi-square decomposition (Sokal and Rohlf 1981). Because many of the genotypes were represented by a single specimen, it was necessary to use the approach of Roff and Bentzen (1989) to estimate the significance of chi-squares computed from the raw data. This Monte Carlo approach was designed for situations where many cells are represented by one or a few individuals.

Results

Restriction endonuclease digestions of mtDNA for 168 *T. chalcogramma*, from three localities in the Bering Sea and one in the Gulf of Alaska, revealed a total of 50 unique restriction sites. All of the nine endonucleases produced variant restriction patterns both within and among populations. *EcoR* I digests yielded the fewest profiles, two, while *Dra* I generated the most, seven. Molecular weight estimates for these fragments are presented in Table 1. The estimated molecular weight for the entire mtDNA molecule was 16.8 ± 0.078 kb (SD). No

TABLE 1. Molecular weight estimates for restriction fragments of *T. chalcogramma* mtDNA produced by the nine endonucleases listed below.

EcoR I		*Sst* II			*EcoR* V		
A	B	A	B	C	A	B	C
8.4	8.4	15.1	9.2	7.0	8.0	5.8	5.8
8.4	5.1	1.7	3.1	6.8	7.1	4.9	4.9
	3.3		2.8	1.7	1.7	2.3	2.3
16.8	16.8		1.7	1.1		2.2	1.7
		16.8	16.8	16.6		1.7	1.3
							0.5
							0.3
					16.8	16.9	16.8

Xba I					*Apa* I				
A	B	C	D	E	A	B	C	D	E
7.5	7.5	7.5	4.7	4.5	6.3	8.5	8.5	8.5	8.5
4.7	4.7	2.5	4.5	3.0	3.8	3.8	3.4	3.8	3.8
2.2	2.6	2.3	3.0	2.5	3.1	3.1	3.1	3.4	1.9
2.1	2.1	2.2	2.2	2.3	2.2	1.1	1.1	1.1	1.2
0.3		2.1	2.1	2.2	1.1	0.3	0.4		1.1
		0.3	0.3	2.1	0.3		0.3		0.3
				0.3	16.8	16.8	16.8	16.8	16.8
16.8	16.9	16.9	16.8	16.9					

Hind III						*Pst* I					
A	B	C	D	E	F	A	B	C	D	E	F
5.6	5.6	5.4	5.4	5.6	5.6	7.1	7.1	7.1	7.1	7.1	7.1
5.4	5.4	4.7	4.7	5.4	5.0	3.7	3.7	3.4	4.8	4.0	3.7
2.9	3.1	2.2	2.9	4.0	2.9	3.4	3.6	2.0	3.7	2.8	2.7
1.8	1.8	1.8	1.8	1.8	1.8	1.5	1.5	1.7	0.9	1.5	1.5
1.1	1.1	1.1	1.1		1.1	0.9	0.9	1.5	0.2	0.9	0.9
		0.6	1.0		0.4	0.2		0.9		0.5	0.7
		0.7						0.2			0.2
16.8	17.0	16.5	16.9	16.8	16.8	16.8	16.8	16.8	16.7	16.8	16.8

Bgl I						*Dra* I						
A	B	C	D	E	F	A	B	C	D	E	F	G
7.2	7.2	10.5	6.3	6.3	6.3	6.8	6.8	6.8	6.8	6.8	6.8	5.7
6.3	5.1	6.3	5.9	4.3	4.5	3.4	6.2	3.4	4.0	5.0	3.4	3.4
3.3	3.3		3.3	2.9	2.7	2.8	2.5	3.1	2.0	2.5	2.5	2.8
	1.1		1.3	2.3	2.3	2.5	0.7	2.8	1.4	1.4	2.3	2.5
				1.0	1.0	0.7	0.6	0.7	1.3	1.2	0.7	1.1
						0.6			0.7		0.6	0.7
									0.5		0.5	0.6
16.8	16.7	16.8	16.8	16.8	16.8	16.8	16.8	16.8	16.7	16.9	16.8	16.8

mtDNA length polymorphisms were noted in any of the walleye pollock digests, although they have been detected in a number of other commercially important fish species (Chapman 1987, 1989; Bentzen et al. 1988; Mulligan and Chapman 1989).

The composite mtDNA digestion patterns revealed a dominant genotype (Table 2, No. 4) consisting of 37 restriction sites found in 39 (23%) of the individuals examined. Genotype 5, which differed from genotype 4 by a single *Dra* I site loss was represented by 21 (12.5%) of the surveyed individuals. Genotypes 4 and 5 were most common in the Donut Hole and Bogoslof Island populations, where they represented 54 and 43% (respectively) of the individuals from these areas. Three genotypes, Nos. 10, 38, and 39, each consisted of 10 individuals. Genotype 10 was equally represented by the Bogoslof Island and Gulf of Alaska populations, while genotypes 38 and 39 were most often detected in the Adak Island population. Fifty-one genotypes (78%) were represented by a single specimen.

Estimates of percent nucleotide divergence between populations ranged between 0.215 and 0.539, with the larger values typically associated with comparisons involving the Adak Island population (Table 3). UPGMA clustering of these distances indicated three major groupings or stocks: (1) Donut Hole/Bogoslof Island, (2) Gulf of Alaska, and (3) Adak Island (Fig. 2).

Heterogeneity chi-square decomposition (Table 4) began by estimating the significance of the chi-squares computed from raw data for all four populations. The computed value of 222.51 was exceeded only once in 1000 simulations, with the largest simulated value LSV = 226.73. It can be concluded that mtDNA genotypes are not randomly distributed in these pop-

TABLE 2. Mitochondrial DNA genotypes observed in *T. chalcogramma*. Numerals indicate number of individuals composing each genotype. Letter sequences from left to right refer to the digestion profiles for the nine variable endonucleases: (1) *EcoR* I, (2) *Sst* II, (3) *EcoR* V, (4) *Xba* I, (5) *Apa* I, (6) *Hind* III, (7) *Pst* I, (8) *Bgl* I, (9) *Dra* I.

	Genotype	Gulf of Alaska	Donut Hole	Bogoslof Island	Adak Island
1	AAAAAAAAA	2	1	1	0
2	AAAAAAAAB	0	1	0	0
3	AAAAAAADB	0	0	1	0
4	AAAABAAAA	7	16	12	4
5	AAAABAAAB	3	11	6	1
6	AAAABAAAD	0	1	0	0
7	AAAABAAAE	0	0	1	0
8	AAAABAAAF	0	0	0	1
9	AAAABAABB	1	0	0	0
10	AAAABAACA	4	2	4	0
11	AAAABAACB	2	1	1	0
12	AAAABAACC	1	0	0	0
13	AAAABAACE	0	1	0	0
14	AAAABAAEA	1	0	0	0
15	AAAABAAEB	1	0	0	0
16	AAAABABAB	0	1	0	0
17	AAAABACAA	1	0	0	0
18	AAAABAECE	0	0	0	1
19	AAAABAFAA	0	1	0	0
20	AAAACAAAG	0	0	1	0
21	AAAACACCA	1	0	0	0
22	AAABBAAAB	0	1	0	0
23	AAABBAABA	0	1	0	0
24	AAACACCAB	0	0	0	1
25	AAADBAAEA	0	0	0	1
26	AAADFAACA	0	0	1	0
27	AABAAAAAA	0	1	1	0
28	AABABAAAA	0	1	3	0
29	AABABAAAB	0	1	4	0
30	AABABAABA	0	1	1	0
31	AABABAABB	0	0	1	0
32	AABABAACA	0	0	1	0
33	AABABAADA	0	0	1	0
34	AABABABAB	0	0	1	0
35	AABABADAF	0	0	1	0
36	AACAAAAAA	1	0	0	0
37	AACAABAAA	0	0	0	1
38	AACABAAAA	2	3	0	5
39	AACABAAAB	4	1	0	5
40	AACABAAAC	1	0	0	1
41	AACABAAAG	0	0	0	1
42	AACABAACA	0	0	0	1
43	AACABAADA	0	0	0	1
44	AACABACAB	2	0	0	0
45	AACABBAAA	0	0	0	1
46	AACABCDAC	0	1	0	0
47	AACABDDAA	0	0	0	1
48	AACADAAAB	0	0	0	1
49	AACAECAAB	0	1	0	0
50	AACBBAAAB	1	0	0	0
51	AACCBAAAA	0	0	0	1
52	AACCBAADA	0	0	0	1
53	AACCBCAEA	0	0	0	1
54	AACCCAAAB	0	0	0	1
55	AACDBAAAA	0	0	0	1
56	AACEBAAAA	0	0	0	1
57	AACEBFAAB	0	0	0	1
58	ABAABEACA	0	0	0	1
59	ABACBAAAA	0	1	0	0
60	ABADBAADA	0	0	0	1
61	ABCBBAAAA	0	0	0	1
62	ACCABAAAA	0	1	0	0
63	BAAABAAAA	1	0	0	1
64	BACABAAAA	0	0	0	1
65	BACCBAAAA	0	0	0	1
		36	50	42	40

TABLE 3. Percent nucleotide divergence between populations of *T. chalcogramma* based on mtDNA restriction sites.

	1	2	3	4
1. Gulf of Alaska	0	0.217	0.215	0.426
2. Donut Hole		0	0.260	0.463
3. Bogoslof Island			0	0.539
4. Adak Island				0

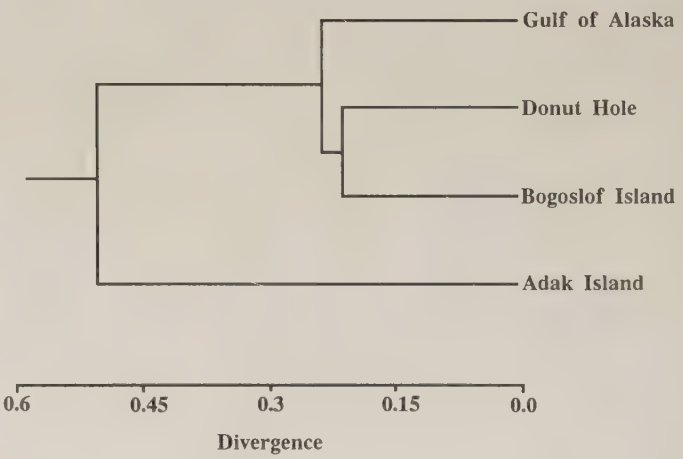

FIG. 2. UPGMA clustering of genetic distances among *T. chalcogramma* populations in the eastern Bering Sea and Shelikof Strait, Gulf of Alaska.

TABLE 4. Results of chi-square tests for homogeneity among and between populations of *T. chalcogramma*. Probabilities and largest simulated chi-squares calculated from the Monte Carlo approach of Roff and Bentzen (1989). Population 1 = Gulf of Alaska, 2 = Donut Hole, 3 = Bogoslof Island, and 4 = Adak Island.

Population comparisons	Chi-square raw data	Probability	Largest simulated chi-square
1, 2, 3, 4	222.51	<0.002	226.73
1, 2, 3	93.29	<0.045	104.94
1, 2, 4	127.95	<0.005	136.51
1, 3, 4	128.01	<0.001	127.06
2, 3, 4	136.45	<0.001	130.35
1, 2	37.13	<0.103	47.39
1, 3	38.75	<0.021	47.12
1, 4	44.13	<0.141	51.56
2, 3	29.03	<0.606	44.58
2, 4	62.36	<0.001	58.31
3, 4	66.56	<0.001	56.93

ulations. Tests in all possible three-way comparisons (Table 4) also indicated significant heterogeneity. Of the six pairwise comparisons, three were found to be significantly different (Adak Island versus Donut Hole and Bogoslof Island; Gulf of Alaska versus Bogoslof Island). These results indicate that mtDNA frequency distributions in Donut Hole and Bogoslof Island populations are more similar to each other than they are to Adak or Gulf of Alaska populations and together constitute a single stock. The Gulf of Alaska can be related to either the Donut Hole or Bogoslof Island area, but Adak Island is significantly different from the other Bering Sea populations. Overall the chi-square results reinforce the UPGMA clusters in Fig. 2.

Discussion

There have been several attempts, using a variety of methods, to define walleye pollock stocks in the North Pacific. In Canadian waters, preliminary tagging (Saunders et al. 1989), parasite (Arthur 1983), and length-at-age frequency data (Thompson 1981; Shaw and McFarlane 1986; Saunders et al. 1989) suggest some discreteness of stocks. Similarly, in the western Pacific, several populations of walleye pollock have been tentatively identified in Japanese waters by morphological (Ishida 1954; Hashimoto and Koyachi 1969, 1971; Iwata 1975a), meristic (Ogata 1959; Iwata and Hamai 1972; Iwata 1975a), and growth (Hashimoto and Koyachi 1977) differences.

The large multinational walleye pollock fishery in the Bering Sea requires that each stock be identified and managed separately. In regard to U.S. Domestic fisheries, the walleye pollock studied here from the Bogoslof Island, Adak Island, and Shelikof Strait areas are within the U.S. 200-mi Exclusive Economic Zone and support significant domestic and/or joint venture walleye pollock fisheries.

Previous work done on the stock structure of walleye pollock in the Bering Sea is summarized in Table 5. In a study encompassing most of the Bering Sea, Serobaba (1975) identified four major stocks of walleye pollock. Eight meristic and 11 morphometric characters were used to arrive at these groupings. Other meristic and morphometric studies have been less informative, as no significant variation was found in several characters examined in walleye pollock from the eastern and central Bering Sea (Dawson 1989) and from throughout the North Pacific (Schultz and Welander 1935; Wilimovsky et al. 1967). Length-at-age and growth differences led Lynde et al. (1986) to describe two discrete stocks in the eastern Bering Sea. Here, Aleutian Basin fish aligned with northern slope and shelf populations. Similarly, using age composition and length-at-age frequency data, Dawson (1989) suggested that Donut Hole fish belong to a broadly defined U.S. Aleutian Basin population which is distinct from an eastern shelf stock. Spawning distributions led Maeda (1972) to conclude that two stocks of walleye pollock exist in the eastern Bering Sea, one north and one south of the Pribilof Islands. A more extensive study, based on temporal and spatial spawning distributions, length-at-age data, and fecundity relationships, suggests that at least three separate spawning stocks of walleye pollock exist in the Bering Sea (Hinckley 1987). These are located in the Aleutian Basin, over the southeastern continental shelf and slope and northwest shelf areas, and over the continental slope northwest of the Pribilof Islands. In the eastern Bering Sea, Mulligan et al. (1989) identified three distinct stocks of walleye pollock based on the elemental composition of otoliths. These were located over the southeast shelf/slope, the northwest shelf, and in the south-central Aleutian Basin. Allozyme studies have been used to delineate walleye pollock from broadly separated areas, i.e. the eastern Bering Sea and Sea of Japan (Iwata 1975a, 1975b), but attempts to identify stocks within the southeastern Bering Sea have been unsuccessful, indicating that these fish compose a single stock or that noninformative loci have been examined (Grant and Utter 1980). In contrast, the mtDNA analysis presented here strongly indicates that at least two genetically discrete stocks exist in the eastern Bering Sea, a Donut Hole/Bogoslof Island group and an Adak Island group.

In regard to the Donut Hole controversy, Dawson (1989) concluded that walleye pollock from throughout the U.S. portion of the Aleutian Basin compose a single stock with fish from this area. Our results partially support this conclusion, as we found strong genetic similarities between the Donut Hole and Bogoslof Island areas and conclude that fish from these areas compose a distinct stock (Fig. 2; Table 3). However, it should be noted that our results are limited in a temporal distribution to 1 yr and should be considered preliminary until further work can determine if interannual variability is less than geographic separation. Although no studies have examined interannual differences in mtDNA genotype frequency, within strictly marine populations, Chapman (1987) noted significant genotypic differences between 1984 and 1986 for striped bass in Chesapeake Bay. In any case, it should be stressed that if temporal differences in mtDNA frequencies do exist in marine populations, this does not indicate that mtDNA is an unreliable indicator of stock structure but rather a tool to reveal unsuspected stock dynamics provided adequate sampling can be done.

In our survey, a comparison of the Donut Hole and Bogoslof Island fish reveals the least nucleotide divergence (Fig. 2), indicating that they belong to the same stock. This is not surprising considering the relatively short distance (approximately 500 km) between these two areas (Fig. 1). Tagging studies indicate that walleye pollock are easily capable of migrating such distances (Takahashi and Yamaguchi 1972). In addition, the movement of fish between these two areas may be facilitated by the counterclockwise gyre system (Kinder et al. 1980; Reed et al. 1988) found along the southeastern Basin (Fig. 3).

Nucleotide divergence estimates also indicate that the Gulf of Alaska and Donut Hole/Bogoslof Island stocks are more closely related to one another than they are to the Adak Island

TABLE 5. Proposed stock units of *T. chalcogramma* in the Bering Sea.

Reference	Technique	No. of stocks	Location
Serobaba 1975	Meristic/morphometric	4	North, south, east, west Bering Sea
Dawson 1989	Morphometric	1	Eastern and central Bering Sea
Lynde et al. 1986	Length-at-age	2	Aleutian Basin and continental slope and shelf north of the Pribilof Islands; continental slope and shelf south of the Pribilofs
Dawson 1989	Length-at-age	2	Aleutian Basin including Donut Hole; eastern shelf
Maeda 1972	Spawning distributions	2	North of Pribilofs; south of Pribilofs
Hinckley 1987	Spawning distribution/ length-at-age/fecundity	3	Aleutian Basin; southeastern continental slope and shelf and northwest shelf; continental slope northwest of the Pribilofs
Mulligan et al. 1989	Otolith composition	3	Southeast shelf and slope; northwest shelf; southcentral Aleutian Basin
Grant and Utter 1980	Allozyme	1	Southeastern Bering Sea
Present study	mtDNA	2	Aleutian Basin; Donut Hole/Bogoslof Island, Adak Island

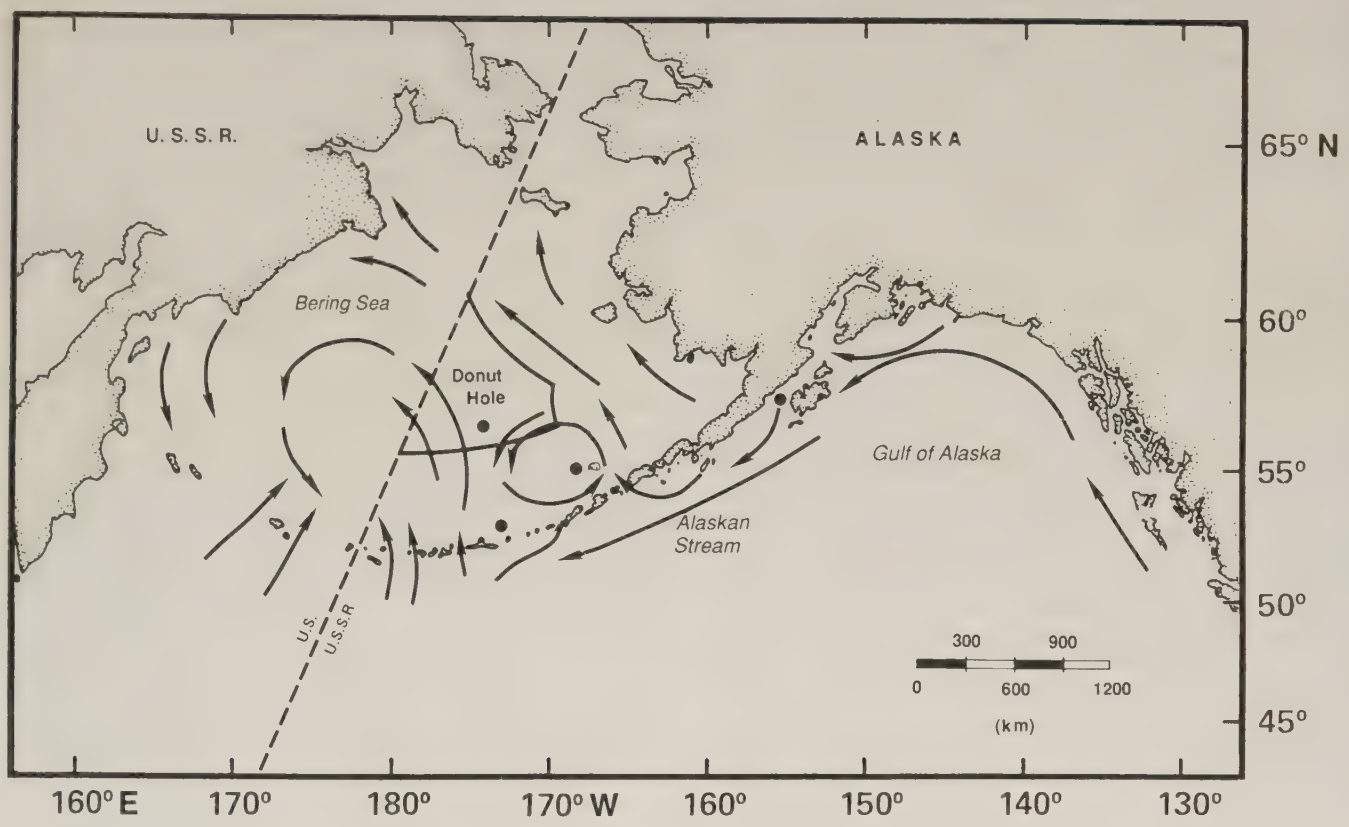

FIG. 3. Surface current patterns and study sites (solid circles) in the Bering Sea and Gulf of Alaska.

population. This may be due to the movement of fish from the Gulf to the Bering Sea via Unimak Pass. As shown in Fig. 3 the strong Alaskan stream flows around the Gulf, from southeast Alaska through and beyond Unimak Pass. This current certainly could influence the southwestward movement of larval and adult walleye pollock from Shelikof Strait through Unimak Pass and eventually to the Bogoslof Island area. Large spawning concentrations of walleye pollock observed in Unimak Pass (Hinckley 1987) indicate that there must be some mixing between these two stocks. Additional evidence of intermingling between fish from these two areas is provided by young-of-the-year surveys, conducted by the Northwest and Alaska Fisheries Center, NOAA, which show juvenile walleye pollock to extend down the Gulf side of the Aleutians and into Unimak Pass. The slight but informative mtDNA differences noted between the Gulf of Alaska and Donut Hole/Bogoslof Island stocks agree with the small allozyme differences observed between Bering Sea and Gulf of Alaska walleye pollock (Grant and Utter 1980).

The greater nucleotide divergence associated with the Adak Island stock (Table 3; Fig. 2) indicates that this stock is moderately isolated from each of the others. It may be a discrete local stock similar to the southern Aleutian stock Serobaba (1975) identified using morphometric and meristic characters. Alternatively, the Adak Island group may be part of an Aleutian Trench or western Bering Sea stock, a scenario implicated by the prevailing currents found in this area of the Aleutian Islands (Fig. 3).

With the exception of work done on Atlantic herring, *Clupea harengus* (Kornfield and Bogdanowicz 1987), studies dealing with intraspecific mtDNA variation within marine fish have utilized very small sample sizes (see Graves et al. 1984; Graves and Dizon 1989; Smith et al. 1989). Consequently, the lack of sequence variation noted in these studies may be due to the limited number of fish sampled. To better understand the stock structure of walleye pollock in the Bering Sea, adequate sample sizes over multiple years and from additional areas must be examined. The great number of genotypes noted in this study (Table 2) further underscores the need of sufficient sampling to properly examine interpopulation variation. If only a few samples from each population had been examined, most of the rare genotypes, i.e. those seen only in a single individual (Table 2), would have gone undetected and may have resulted in different patterns of differentiation. Similar results and conclusions have been noted in Atlantic herring (Kornfield and Bogdanowicz 1987), striped bass (Chapman 1989), and white perch (Mulligan and Chapman 1989). mtDNA analyses involving walleye pollock from throughout the Bering Sea would be ideal but impractical due to the tremendous effort and expense involved in sampling such a vast area. Consequently, future studies should focus on additional areas which support major pollock fisheries, such as in Soviet waters southeast of Cape Navarin and northeast of the Kormandorskie Islands and in U.S. waters from Unimak Island to St. Matthew Island (Fig. 1). It would be especially informative to add to our data set a population from north of the Pribilof Islands. Based on growth differences, Lynde et al. (1986) reported that fish from this area, along with Aleutian Basin fish collected approximately 30 km from the southeastern boundary of the Donut Hole, compose a discrete stock. Regardless of future studies, it is evident from our results that there is sufficient variation in walleye pollock mtDNA to suggest stock discreteness within oceanic waters. Finally, due to the heavy fishing in the international waters of the Aleutian Basin, future studies must verify the uniqueness or the origin of walleye pollock in the Donut Hole

to ensure the proper management of this highly valuable Bering Sea resource.

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Modelling Crustacean Fisheries: Effects of Parasites on Management Strategies

Armand M. Kuris and Kevin D. Lafferty

Department of Biological Sciences, and Marine Science Institute, University of California, Santa Barbara, CA 93106, USA

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The reproductive performance of commercially important crustaceans may be considerably affected by symbiotic nemertean egg predators and parasitic castrators. Because these parasites commonly affect only females or feminize males, they may be protected by management practices that protect females. To manage a parasitized stock, we suggest that strategies should vary depending on the recruitment dynamics of both host and parasite. For a certain spatial scale, recruitment to a population may be "open" or "closed" depending on the behavior of planktonic larvae, the duration of these planktonic stages, and the flush rate of the environment of the adult host. Both hosts and parasites may have open or closed larval recruitment. We developed mathematical models to investigate the impact of protection of females on a hypothetical fishery for different combinations of host and parasite recruitment dynamics. The models suggest that the common practice of releasing females is not advantageous when a fishery is affected by a parasite. Retaining females in the catch is preferable in most cases. Treating or culling infected females may be advisable when host recruitment is closed.

Le succès de la reproduction d'espèces de crustacés d'importance commerciale peut être fortement compromis par des némertiens symbiotes prédateurs des oeufs et des «castrateurs» parasites. Étant donné que ces parasites n'attaquent ordinairement que les femelles et féminisent les mâles, ils peuvent être protégés par les mesures de gestion visant la protection des femelles. Les auteurs recommandent que les stratégies de gestion de stocks parasités devraient être adaptées à la dynamique du recrutement de l'hôte et du parasite. à une certaine échelle spatiale, le recrutement à une population peut être «ouvert» ou « fermé» selon le comportement des larves planctoniques, la durée de ces stades planctoniques et le taux d'élimination de l'environnement de l'hôte adulte. Les hôtes et les parasites peuvent aussi avoir un recrutement de larves ouvert ou fermé. Les auteurs ont élaboré des modèles mathématiques pour étudier l'impact de la protection des femelles sur une pêcherie hypothétique en fonction de diverses combinaisons de la dynamique du recrutement des hôtes et des parasites. Les modèles portent à croire que la pratique commune de remettre les femelles à l'eau n'est pas avantageuse lorsqu'une pêcherie est compromise par un parasite. Ils sont d'avis que ces femelles ne devraient pas être remises à l'eau dans la plupart des cas. Le traitement ou la destruction sélective des femelles infectées peut être indiqué lorsque le recrutement de l'hôte est fermé.

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Infectious organisms may affect growth (Overstreet 1979; Lester 1978b; Sloan 1984), reproduction (Sloan 1984; Lester 1978a; Hicks 1982), egg survival (Wickham 1979; Kuris and Wickham 1987; Campbell and Brattey 1986; Shields et al. 1990a; Kuris 1992), longevity (Meyers et al. 1989), and marketability (Meyers et al. 1989; Sparks and Hibbits 1979) of commercially important Crustacea. In some cases, the infectious agents are abundant and may significantly affect host populations (Botsford and Wickham 1978; Botsford 1986; Hobbs and Botsford 1989; Shields et al. 1990b; Kuris et al. 1991). In other situations, insufficient data are available to conclude that host population dynamics are affected, but the infectious organisms are sufficiently prevalent to suggest so (Sloan 1984; Sparks 1982).

Several distinctive host–parasite interactions may be important to fisheries. Microbial diseases, which generally reproduce to high intensities within a host, include bacteria (e.g. Stewart et al. 1966), fungi (e.g. Sparks 1982), protozoans (e.g. Newman and Ward 1973; Meyers et al. 1989; Skurdal et al. 1990), and viruses (e.g. Couch et al. 1975; Johnson 1983). The population dynamics of such "microparasites" (sensu Anderson and May 1979) have been extensively investigated (Anderson and May 1979, 1981; May 1985) with some consideration given to finfish fisheries (Dobson and May 1987).

Parasitic castrators, such as rhizocephalan barnacles and epicaridean isopods, block the reproductive output of their host (Kuris 1974). Male hosts are frequently feminized morphologically and behaviorally (e.g. Veillet 1945; Reinhard 1956; Rasmussen 1959; Phillips and Cannon 1978). Unlike microparasites, the pathological effects of parasitic castrators are not additive; an individual castrator completely prevents host reproduction (Kuris 1974).

Nemertean worms feed on embryos of decapod crustaceans (Humes 1942; Kuris 1978; Wickham 1979; Aiken et al. 1985; Wickham et al. 1985, Shields et al. 1989; Shields and Kuris 1990). These have been termed symbiotic egg predators (Kuris 1971, 1992; Kuris and Wickham 1987). At high intensities, nemerteans can kill more than half the eggs produced by host populations (Wickham 1979, 1986; Shields and Kuris 1988; Kuris et al. 1991). They have been included in models of the cyclic dynamics of the Dungeness crab, *Cancer magister*, fish-

ery in California (Botsford and Wickham 1978; Botsford 1986; Hobbs and Botsford 1989).

Standard management practices on commercial crustacean species, including restrictions on catch (number of individuals), size (minimum length or width), sex (males only), effort (number of fishing days), location, seasons (usually nonbreeding), and gear, are usually ad hoc measures aimed at protecting the reproductive output of the stock (Botsford 1992). Each of these practices may increase the overall impact of parasites on future yield if fishing increases the relative abundance of parasitized hosts. This may occur when parasitized individuals grow more slowly (Hicks 1982), females are not fished but the infectious agent occurs predominantly on females (e.g. Lester 1978a; Wickham et al. 1985; Shields et al. 1990b; Kuris et al. 1991), changed behavior of parasitized individuals reduces their rate of entry to traps (Crothers 1968; Sloan 1984; Skurdal et al. 1990), heavily parasitized geographic locations are less exploited by fishermen (Hicks 1982), or infected individuals are released following shipboard culling because they are unmarketable (Petrushevski and Schulman 1958).

Management is essential for conservation and perpetuation of crustacean fisheries. We argue that infectious organisms substantially impact some important crustacean fisheries and that they should be considered in management strategies. Our argument is based on a simple fisheries model in which we incorporate parasites and then vary management practices and recruitment dynamics of both host and parasite.

Recruitment Dynamics

To manage a parasitized stock, we propose that fishing practices should vary depending on the recruitment dynamics of both the host and parasite. Recruitment to a stock may be conceived as comparatively "open" or "closed" (sensu Caswell 1978) based on the relative spatial scales of the fishery compared with host and parasite population dynamics. For the host, this depends on the behavior of planktonic larvae, duration of larval stages, and the flush rate of the water mass of the adult habitat. In open recruitment systems, larval dispersal is widespread and postlarval settlement is not dependent on productivity of adults from that location. In closed systems, larval dispersal is relatively confined and settlement is dependent on productivity of the adults from that location. Larval duration is probably the most useful and available clue for appropriate classification of the spatial scale of host recruitment dynamics.

For the parasites, the mode of transmission and infection dynamics are the principal determinants of the scale of parasite recruitment. Closed recruitment will likely result if the infectious organism is transmitted by contact between infected and uninfected hosts (including cannibalism), the free-living infectious stage is short lived or limited in its mobility, or the movement of larvae between water masses is restricted geographically (e.g. Sloan 1985).

The scale of recruitment patterns has attracted increasing attention with respect to theory (Roughgarden et al. 1988; Warner and Hughes 1988; Underwood and Fairweather 1989; Possingham and Roughgarden 1990) and its application to fisheries (Dadswell 1979; Harding et al. 1983; Ennis 1986). As most benthic crustaceans important to fisheries have long-lived larvae, it is generally assumed that the source of larvae for recruitment is not locally based (Jamieson 1986). However, for the American lobster, *Homarus americanus*, the crustacean for which there are the most data on geographic abundance and movement of larvae, the nature of recruitment patterns continues to be actively debated (e.g. Ennis 1986).

Classical population theory is based on the assumption of a closed system, and Dobson and May (1987) modelled the effects of parasites on fisheries in this context. However, the likelihood that the scale of parasite recruitment may differ from that of the host introduces unexpected complexity to host–parasite recruitment dynamics. Figure 1 illustrates the 2 × 2 contingency matrix for open versus closed recruitment of hosts and parasites. We speculate that parasite recruitment will often be closed relative to the recruitment pattern of the host. Comparatively few parasites of crustaceans appear to have long-lived, widely dispersing transmissive stages. For example, the rhizocephalans, an important group of parasitic castrators (Kuris 1974), have free-living transmissive stages (nauplii, cypris) that are nonfeeding and usually competent to infect a new host in a few days (Høeg and Lützen 1985).

A likely example of a parasite closed – host open recruitment system is the interaction between the nemertean egg predator, *Carcinonemertes regicides*, and its host, the red king crab, *Paralithodes camtschaticus*, in the Kodiak Archipelago and in southeastern Alaska (Fig. 1). Parasite abundance varies greatly on a small geographic scale and, although transmission has not been directly studied, the temporal pattern of rapid increase on infected hosts indicates that the free-living stage is so brief that autoinfection has been postulated (Kuris et al. 1991). In contrast with the parasite, the host's larvae develop over a 3- to 4-mo period (Weber 1967), and substantial larval transport over considerable distances appears probable for some areas (Haynes 1974).

A system in which recruitment of both host and parasite is closed is the golden king crab, *Lithodes aequispina*, and the rhizocephalan, *Briarosaccus callosus*, in British Columbia (Fig. 1). Some populations are confined in isolated fjords where sills near the mouths restrict water movement (Sloan 1984, 1985). Host larval behavior (e.g. hatching at depth) enhances the likelihood of retention. Parasite larvae are released in the same habitat (Sloan 1985). Their retention is further enhanced by a relatively short larval duration (Hawkes et al. 1985). Another closed–closed system occurs with crayfish, *Astacus astacus*, and a microsporidian. Crayfish lack a planktonic larval stage and the microsporidian, *Thelohania*, is transmitted by cannibalism (Skurdal et al. 1990).

An example of open host and parasite recruitment is the Dungeness crab fishery in northern California which is heavily infested with *Carcinonemertes errans* (Wickham 1986) (Fig. 1). Larval development of Dungeness crabs is long (3-4 mo, Reilly 1983), and exposure to strong currents prevents the separation of distinct populations (Soulé and Tasto 1983). Although larval settlement of *C. errans* has not been achieved in laboratory studies, preliminary evidence indicates a lengthy larval period of more than 50 d (Roe 1979).

Examples of host closed – parasite open recruitment patterns might be expected in situations where crustaceans act as intermediate hosts and definitive hosts disperse parasite eggs widely (Kuris 1990). This may occur with the Chinese lung fluke, *Paragonimus westermani*, a serious human parasite, that uses the Chinese mitten crab, *Eriocheir japonicus*, and other freshwater crustaceans as intermediate hosts. Humans and other vertebrates become infected by eating raw crabs that may be parasitized at a prevalence (the proportion of hosts infected, Margolis et al. 1982) of 70% (Yokogawa 1953). These larval trematodes are "typical parasites" (sensu Kuris 1974).

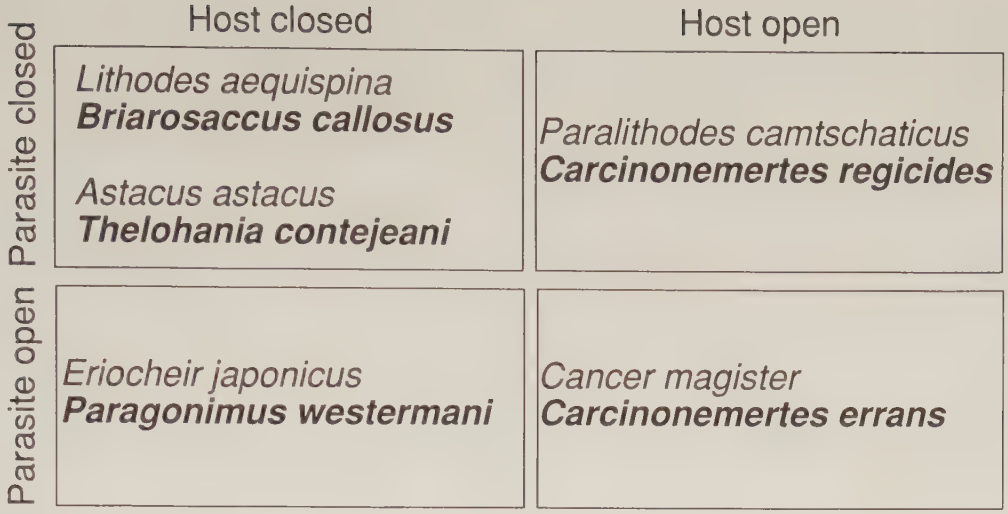

FIG. 1. Interaction between the scale of host and parasite recruitment dynamics presented as a 2 × 2 contingency table. Examples of fisheries in these categories are provided.

Although there may be potential for these parasites to affect crabs, control of lung flukes is directed at reducing transmission to humans. Our general recommendations do not address this sort of public health issue.

Table 1 presents a list of important crustacean fisheries for which there is substantial evidence that infectious diseases may significantly affect the yield or reproductive output of at least some stocks. Unfortunately, the relative scales of recruitment for host and parasite are not known for most of the examples and they cannot, therefore, be placed in the recruitment categories of Fig. 1.

We emphasize that open and closed recruitment are relative terms, although we treat them in an absolute sense for modelling purposes. The most readily recognizable systems are those in which the recruitment pattern of the host strongly contrasts with transmission dynamics of the parasite (host open – parasite closed, host closed – parasite open). The distinction between host open – parasite open and host closed – parasite closed depends on the scale of recruitment relative to that of the fishery. For example, a fishery that exploits a small geographic region of a closed host population might be best managed as an open system if recruits to the fished area were independent of the parent stock in that area.

The Model

Efforts to diminish the impact of a parasite on a fishery may benefit from the incorporation of epidemiological theory in fisheries models. The idea that a reduction in host abundance below a certain threshold will eliminate a parasite was first argued analytically by Kermack and McKendrick (1927). Dobson and May (1987) proposed the same mechanism for finfish fisheries.

Here, we present a model that compares the effects of various management strategies on a hypothetical crustacean fishery that is parasitized by a parasitic castrator. The model is developed for the four recruitment patterns of host and parasite depicted in Fig. 1; we have not attempted to simulate a specific system.

We employed difference equations to subdivide a crustacean host stock into five life history stages (larvae, juveniles, males, uninfected females, and infected females). Our comparisons are based on stock sizes and fishery yields at equilibrium levels. For this reason, the models were deterministic and deliberately stabilized by density-dependent natural mortality.

Assumptions

To specifically investigate crustacean fisheries, we assumed that the host reproduces seasonally and the parasite impacts reproduction (not mortality) and we distinguish fishing mortality for both male and female hosts.

We assumed that per capita natural mortality of infected and uninfected hosts (adults and juveniles) was dependent on the density of hosts (with juveniles using one half the resources of adults). Per capita larval mortality and fishing mortality, on the other hand, were assumed to be density independent.

The parasite population was represented by the prevalence of infected hosts. In the model, parasites (actually parasitic castrators) eliminated the fecundity of infected females (although we believe that egg predators and microparasites will have similar effects, these parasites cause only partial reduction in host fecundity and, for egg predators, induce intensity-dependent pathology and have an aggregated distribution). Parasite mortality occurred only when infected hosts died or were treated. If the parasite affects both marketability and reproduction, its impact on the fishery will be greater than we calculate. This would be especially true for cases where host recruitment is open.

The parasite was assumed to be host specific. If the parasite is not host specific, fishing the target species would have a lessened effect on prevalence. Petrushevski and Schulman (1958) suggested, in this case, that efforts should be made to harvest alternative hosts as well.

We assumed that fertilization rates for females were independent of sex ratio and that the sex ratio of recruits was 1:1. Recruitment of host or parasite was defined as either open or closed. Where host recruitment was closed, larval density was a linear function of the density of adult, uninfected females. Where the recruitment of the host was defined as open, larval density was constant (in nature this would be a stochastic parameter). Where parasite recruitment was closed, parasite transmission was a linear function of the product of infected

TABLE 1. Some infectious agents for which there is evidence that recruitment, yield, or marketability of commercially important crustacean hosts was substantially affected. Prevalences reported are peak values.

Fishery	Region	Parasite	Interaction	Prevalence (%)	Reference(s)	Remarks
Lithodes aequispina	British Columbia	*Briarosaccus callosus* (Rhizocephala)	Parasitic castrator	40	Sloan 1984, 1985	Parasitized hosts behaved like postspawned female hosts. Host and parasite are continuous spawners; had reduced growth
Paralithodes camtschaticus	Alaska	*Carcinonemertes regicides* (Nemertea)	Symbiotic egg predator	100	Kuris et al. 1991	1983–85 caused brood failure in Cook Inlet, around Kodiak I., and in southeastern Alaska; autoinfection
Portunus pelagicus	Queensland	*Sacculina granifera* (Rhizocephala)	Parasitic castrator	12–50+	Lester 1978a; Bishop and Cannon 1979	Parasitized crabs did not molt; behaved like females
Callinectes sapidus	Gulf of Mexico	*Loxothylacus texanus* (Rhizocephala)	Parasitic castrator	?	Overstreet 1978	Affects growth, epizootics in 1965 and 1977
	Virginia	*Paramoeba perniciosa* (Sarcodina)	Microparasite	57	Newman and Ward 1973; Johnson 1977; Couch 1983	"Grey crab disease" always fatal; seasonal epizootics are a frequent cause of mass mortalities
Carcinus maenas	Eastern Atlantic, Mediterranean	*Sacculina carcini* (Rhizocephala)	Parasitic castrator	25–100	Crothers 1968; Høeg and Lützen 1985	Parasitized crabs behave like reproductive females
Cancer magister	California	*Carcinonemertes errans* (Nemertea)	Symbiotic egg predator	100	Wickham 1979, 1986	Can cause brood failure at high intensities
Chionoecetes bairdi	Alaska	cf. *Haemotodinium* sp.(Dinoflagellida)	Microparasite	?	Meyers et al. 1989	"Bitter crab disease"; infected crabs not marketable; fatal
		Trichomaris invadens (fungi)	Microparasite	75	Sparks and Hibbits 1979; Sparks 1982; Hicks 1982	"Black mat disease"; affects population dynamics, growth reproduction, and marketability
Homarus americanus	North Atlantic	*Aerococcus viridans* (bacteria)	Microparasite	40	Stewart et al. 1966; Stewart 1975	"Gaffkemia"; epizootics reported; infection via damaged cuticles; mortality in shipping
Penaeus duorarum	Gulf of Mexico	*Baculovirus penaei* (virus)	Microparasite	80	Couch and Courtney 1977; Couch et al. 1975	Mortality increases with pollutant stress; transmission postulated via cannabilism

female density and uninfected female density. Finally, where parasite recruitment was open, parasite transmission was a linear function of the density of adult, uninfected females.

Per capita fishing mortality, defined as the proportion of adults fished (juveniles were not caught), was independent of density. The yield of the fishery was, therefore, the product of the fishing mortality and the size of the fished population.

Model Structure (see Fig. 2)

(1) If both host and parasite recruitment are closed, the life history stages of the host are represented as

$$(1a) \quad L_{t+1} = sbF_t$$

where L_{t+1} is the number of larvae at time $t + 1$, s is the proportion of larvae that survive to settlement, b is the number of larvae produced per uninfected female, and F_t is the number of uninfected females at time t,

$$(1b) \quad J_{t+1} = (1 - d_t)L_t$$

where J_{t+1} is the number of juveniles at time $t + 1$ and d_t is the density-dependent per capita natural mortality at time t,

$$(1c) \quad M_{t+1} = (1 - d_t)(1 - f_m)(M_t + J_t/2)$$

where M_{t+1} is the number of males at time $t + 1$ and f_m is the proportion of the males taken by the fishery,

$$(1d) \quad F_{t+1} = (1 - d_t)(1 - f_f)(F_t + J_t/2 - (\beta_1 L_t(F_t + J_t/2))$$

where f_f is the proportion of the females taken by the fishery, β_1 is the number of uninfected hosts that become infected by the presence of one infected host from t to $t + 1$ (transmission efficiency), and I_t is the number of infected females at time t, and finally,

$$(1e) \quad I_{t+1} = (1 - d_t)(1 - f_f)(I_t + (\beta_1 I_t(F_t + J_t/2)).$$

The yield to the fishery is presented in three parts, Y_{Mt+1}, Y_{Ft+1}, and Y_{It+1} where Y_{t+1} is the yield at time $t + 1$ (specified by life history stage):

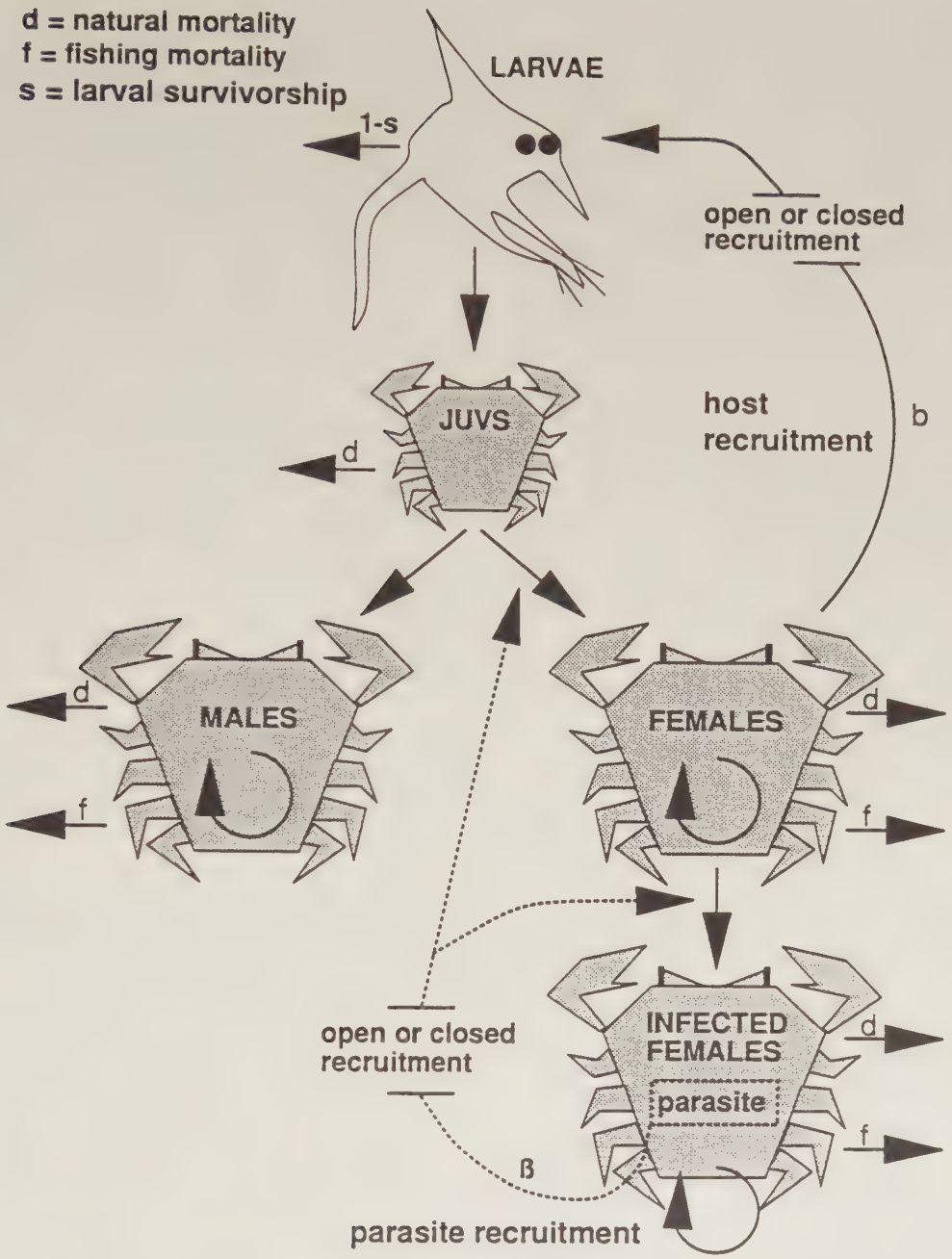

FIG. 2. Flowchart representation of the life history stages – infectious dynamics model. Boxes represent abundances of five life history stages of a crustacean host. Solid arrows represent the movement of individuals into and out of these stages. Broken arrows represent the effect of parasites on the infection status of females and juveniles. Infected females do not reproduce.

(1f) $Y_{Mt+1} = (M_t + J_t/2)f_m(1 - d_t)$

(1g) $Y_{Ft+1} = (1 - \beta_1 I_t)(F_t + J_t/2)f_f(1 - d_t)$

(1h) $Y_{Ii+1} = (I_t + \beta_1 I_t(F_t + J_t/2))\, f_f\,(1 - d_t)$.

Natural mortality is a function of stock size such that

(1i) $d_t = (M_t + F_t + I_t + J_t/2)/K$

where K is a constant that is proportional to the carrying capacity.

(2) If parasite recruitment is open and host recruitment is closed, all the equations in part 1 apply except that

(2a) $F_{t+1} = (1 - d_t)\,(1 - f_f)(1 - \beta_2)(F_t + J_t/2)$

where β_2 is the proportion of uninfected hosts that become infected over time t. Furthermore,

(2b) $I_{t+1} = (1 - d_t)(1 - f_f)(I_t + \beta_2(F_t + J_t/2))$

(2c) $YF_{t+1} = (1 - \beta_2)\,(F_t + J_t/2)f_f\,(1 - d_t)$

(2d) $YI_{t+1} = (It + \beta_2(F_t + J_t/2))\, f_f\,(1 - d_t)$

(3) If host recruitment is open and parasite recruitment is closed, all the equations in part 1 apply except that

(3) $L_{t+1} = L_t$.

(4) If both host and parasite recruitment are open, all of the equations in part 1 apply except those modifications made in parts 2 and 3.

Management Strategies

We investigated the following four management strategies: (1) all adult individuals were kept by the fishery ("keep females"), (2) only adult males were kept by the fishery ("release females"), (3) only adult males were kept by the fishery and infected females were not released ("cull infected females"), and (4) only adult males were kept by the fishery and infected females were treated before they were released ("treat infected females"). These were integrated into the model with the following modifications: keep females, $f_f = f_m$; release females, $f_f = 0$; cull infected females, $f_f = f_m$, $F^*_{t+1} = F_{t+1} + (1 - d_t/2)YF_{t+1}$; and treat infected females, $f_f = f_m$, $F^*_{t+1} = F_{t+1} + (1 - d_t/2)(YF_{t+1} + YI_{t+1})$.

The carrying capacity of the host in the absence of parasites was set to equal 1000 individuals. Simulations were run using an initial equilibrium of 100, 200, or 500 infected females (figures show 200). fishing mortality was then varied from 0 to 1 and yield and the abundance of males, uninfected females, and infected females were recorded for the four management strategies described above.

Results

The effects of management strategies or fishing tactics on yield (in number of marketable individuals), stock size, sex ratio, and parasite prevalences are presented in Fig. 3 and 4. The outcomes are varied and suggest that strong interactions between management, recruitment, and parasites are likely to occur.

No Parasites

To allow a comparison with other published models and clearly demonstrate the effect of parasites, we first present the behavior of the model in the absence of parasites.

Closed host recruitment

Keeping females produced dynamics similar to classic surplus production models (Ricker 1954). This tactic generated a comparatively higher yield at low fishing mortality than did releasing females, due, primarily, to the inclusion of both sexes in the catch (Fig. 3A). The reduction in stock size, however, was more dramatic because recruitment was lower. Maximum sustainable yield, MSY, occurred at an intermediate level of fish mortality where stock size is reduced to half the carrying capacity. With high fishing mortality, both stock and yield declined to zero (sex ratio remained equal).

There was a positive correlation between fishing mortality and yield when females were released (Fig. 3A). This caused a marked decrease in the proportion of males and a slight drop in stock size. If we had assumed that fertilization rate decreased as sex ratio became strongly biased towards females, yield would have declined at sufficiently high fishing mortality.

Open host recruitment

Abundance decreased with increasing fishing mortality, but fishing did not affect the availability of new recruits. For this reason, yield increased with fishing mortality whether females were kept or released (Fig. 3C and 3D). Yield was over twice as high if females were kept than if they were released because both sexes were included in the catch and the productivity of the stock increased at low population densities.

Parasites present

Closed host recruitment

If host recruitment was closed, parasites reduced stock abundance. The percent reduction in host abundance was equal to the percent of individuals that were castrated. This is analogous to results of analytical models of parasitic castrators (Lafferty 1991). Under these conditions, releasing females returned a consistently low yield, even if fishing mortality was high (Fig. 4B and 4F). This was caused by an increase in the abundance of infected females and a subsequent decrease in the abundance of uninfected females. Keeping females, on the other hand, reduced the prevalence of the parasite.

If parasite recruitment was closed, at intermediate levels of fishing mortality, prevalence fell to zero and the system reverted to the hump-shaped trajectory for the yield curve in the absence of parasites (Fig. 4A and 4E). Both culling or treating infected females reduced prevalence to zero at an intermediate fishing mortality. The system then followed the trajectory for releasing females in the absence of parasites (Fig. 3B and 3D).

Open parasite recruitment (Fig. 4E–4H) produced the lowest yields because no fishing strategy can completely eliminate the parasite, and recruitment to the stock is dependent on uninfected female host abundance. Keeping females drove host stock size and yield to zero at moderate fishing mortality. Releasing females provided yields that increased slowly with increasing fishing mortality. Culling infected females generated a higher yield than did releasing females because more resources were made available to the stock. Treating infected females produced the highest yield due to the corresponding increase in reproductive output.

Open host recruitment

If host recruitment was open, parasitism had no effect on the yield of the stock (Fig. 4I–4P). Culling infected females generated a slightly higher yield than either treating infected females or releasing females due to the small increase in productivity associated with lowered stock size.

Fishing affected parasite prevalence. If parasite recruitment was closed, keeping females reduced parasite prevalence as fishing mortality increased, ultimately eliminating the parasite from the stock. Releasing females, under the same conditions, had the opposite effect; this led to greater than 50% prevalence at high fishing mortality. In contrast, if parasite recruitment was open, releasing females led to a slight increase in parasite prevalence, while keeping females did not affect prevalence.

Discussion

For closed systems, Dobson and May (1987) suggested that increasing fishing mortality could eliminate a parasite without causing the collapse of the fishery if the transmission threshold was above the MSY. This assumes that a fishery takes all individuals with equal probability. Parasites of crustaceans, however, often affect only females (e.g. nemertean egg predators) or feminize male hosts (e.g. Rhizocephala). Such hosts may be protected by ad hoc management practices (especially if reproductive output declines). In this case, our model indicates that increased fishing mortality will increase the impact of a parasite. For fishing pressures to reduce parasitism, parasitized individuals must be kept, culled, or treated and released. Which strategy will be most effective depends partially on the recruitment dynamics of the host and parasite. These same arguments apply in any case where a fishery takes a disproportionately

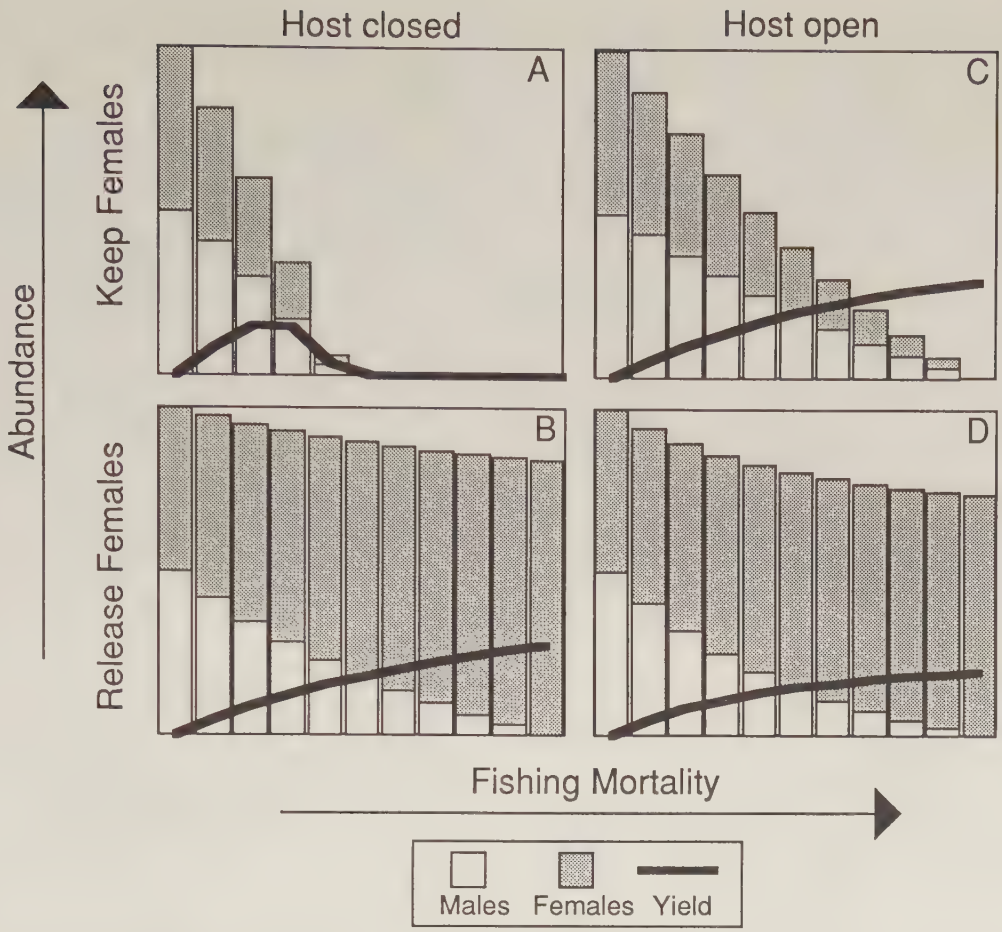

FIG. 3. Fishery yield (solid lines) and the abundance of males (open bars) and females (shaded bars) as a function of fishing mortality for closed and open recruitment when the fishery releases or keeps females.

large percentage of uninfected hosts due to size limits, altered behavior, etc.

Our model limits its investigation of parasitism to female castration. If males are also castrated, the impact of parasitism on the fishery would be higher if infected males were normally released by the fishery, fertilization were dependent on uninfected male density, or if castrated males copulated unproductively with uninfected females. In these cases, the strategy that we suggest for dealing with infected females should also be applied to infected males.

Because stocks may be linked by an exchange of larvae, our modelling of stocks as isolated entities is oversimplified. For example, although parasites will not affect recruitment if host recruitment is open, they may indirectly impact distantly connected stocks that are dependent on a shared source of recruits. This calls for careful evaluation of associated stocks prior to the initiation of a management strategy. A series of connected open systems might be best managed as a single closed system. If variation in parasite prevalence exists between these systems, an adaptive management approach might be most appropriate. In this case, regions with high prevalence of the parasite might be fished with removal of infected hosts. If recruitment of the parasite is locally closed, it may be possible to reduce the impacts of the parasite without significantly reducing the larval pool shared by the connected host stocks. If parasite recruitment is open (within a stock), fishing infected hosts will reduce the export of parasites into other connected stocks.

We note that certain crustacean fisheries are suitable for experimental manipulations to test theoretical predictions and improve yield or recruitment. Many crab and lobster fisheries protect reproductive capacity by releasing females or, at least, ovigerous females, and undersized individuals. Most modern fisheries are sufficiently intensive that a very high proportion of the annual recruits that exceed the fishable size threshold are caught in each fishing season (e.g. Ennis 1986; Jamieson 1986). Consequently, a considerable proportion of the females and small males in these fisheries are also briefly handled and released each season. This is a unique feature of these fisheries that enables experimental intervention.

The Alaskan red king crab fishery is infested with nemertean egg predators in the Kodiak, Cook Inlet, and Southeastern Management Areas, and certain fisheries experience substantial parasitization by rhizocephalans (Table 1). These cases may be suitable for a large-scale manipulation. Experiments could test the hypothesis that diseases affecting reproductive output are an important determinant of subsequent recruitment to the fishery. In practical terms, such an experimental probe may determine whether local stocks can recover. Preconditions for experimentation include classification of the scale of recruitment for both host and parasite as relatively open or closed, determination of the geographic boundaries of closed cells, and cooperation of the commercial fishing industry. Knowledge of the dispersion pattern of parasites among the host populations, of

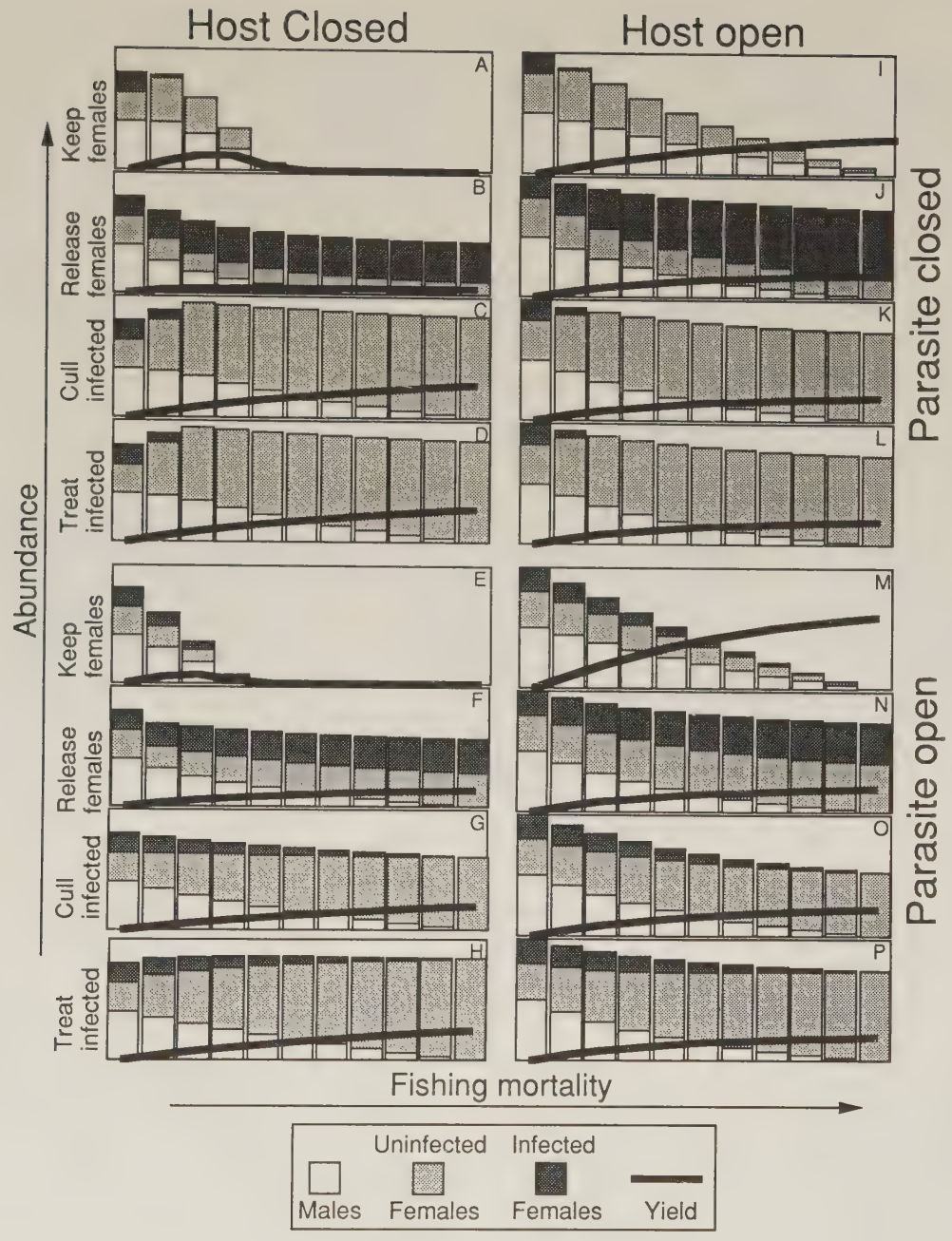

FIG. 4. Fishery yield (solid lines) and the abundance of males (open bars), uninfected females (light shaded bars), and infected females (dark shaded bars) as a function of fishing mortality when the fishery releases females, keeps females, culls infected females, or treats infected females. Yields are indicated for closed and open recruitment of host and parasite.

the use of alternative host species by the parasite, and of seasonality of parasite transmission would aid implementation.

For the Alaskan red king crab, the complex geography of the Alaskan coastline provides an appropriate setting to attempt the substantial reduction of nemertean infestations. Extensive long-term observations by the Alaska Department of Fish and Game and U.S. National Marine Fisheries biologists provide a historic background for catch statistics and reproductive performance in local areas (Blau 1983, 1986, unpublished information), and data are now available for nemertean abundance in these areas (Shields et al. 1990b; Kuris et al. 1991). As this appears to be a host open – parasite closed recruitment system (Fig. 1), our model suggests that retaining females in the catch in locations such as Uganik Bay, Kachemak Bay, Excursion Inlet, or Port Frederick should reduce the abundance of *C. regicides*. The survey of nemertean abundance by Kuris et al. (1991) and Shields et al. (1990b) indicates that there are sufficient areas to replicate treatments. Representatives of commercial fishermen's associations see this as an opportunity to actively improve the depressed red king crab fishery and have expressed a willingness to cooperate with this sort of large-scale experiment.

Fisheries with a high prevalence of rhizocephalan parasitic castrators also provide circumstances suitable for experimentation. For golden king crabs, isolated in fjords of British Columbia and Alaska, a suitable experiment would be to cull

parasitized crabs. A more extensive survey of rhizocephalan distribution and abundance would be needed to provide enough locations for replication.

Reviewing the other fisheries in Table 1, it is evident that more basic research on the epidemiology of these parasites is needed to define the scale of parasite recruitment. This need is greatest for the microparasites, as we are unable to characterize recruitment for most of these infectious agents. Further work on parasite systematics (using molecular techniques) and experimental transfer studies will improve our knowledge of host specificity. Field experiments with model systems, perhaps using intertidal invertebrates, might help test experimental methodologies and prove easier to estimate productivity, recruitment, and other population parameters (Shields and Kuris 1988; Lafferty 1991). Improved understanding of the relationships between host reproductive output, larval abundance, larval behavior, and settlement will also aid the recognition of the relative geographic scale of host and parasite recruitment.

In summary, our model suggests that management strategies should vary based on the recruitment dynamics of host and parasite. Females should be fished if host recruitment is open, or both host and parasite recruitment are closed and fishing effort is moderate, or parasites are unimportant, fishing effort being moderate. Parasitized females should be culled if both host and parasite recruitment are closed and fishing effort is high. Parasitized females should be treated (assuming this is possible) if host recruitment is closed and parasite recruitment is open. Finally, females should be released when parasites are unimportant, fishing effort is high, and host recruitment is closed.

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Coarse Particulate Detritus Dynamics in Small, Montane Streams of Southwestern British Columbia

John S. Richardson[1]

Ecology Group, Department of Zoology, University of British Columbia, Vancouver, B.C., Canada V6T 1W5

Richardson, J. S. 1992. Coarse particulate detritus dynamics in small, montane streams of southwestern British Columbia. Can. J. Fish. Aquat. Sci. 49: 337–346.

The dynamics of coarse particulate organic matter (CPOM) were studied for 2 yr in three second-order streams in the Coast Range of British Columbia. Estimates of direct litterfall ranged from 201 to 481 g ash-free dry mass·m $^{-2}$·yr^{-1}. The magnitude of deciduous leaf litter input was similar among streams. Input of conifer needles to a stream with an old-growth canopy was higher than in two streams which drained second-growth forests. There was over a 60-fold seasonal change in deciduous leaf standing stock, but woody debris and total CPOM showed less seasonal variation. Decomposition of alder leaf packs in two of the streams showed a large temperature-dependent component and significant differences between streams, with the more retentive stream having lower rates of decomposition. From estimates of input and decay rates, models of leaf loss were made to predict benthic standing stocks of deciduous leaf litter. Comparisons of the model predictions with actual measures indicate that 70–94% of leaf material was unaccounted for and presumably lost from the study reach by export, floodplain deposition, and burial. The seasonal changes in standing stock of CPOM emphasize the variation in food supply potentially available to detritivorous stream organisms.

La dynamique de la matière organique sous forme de particules grossières dans trois cours d'eau de second ordre de la chaîne côtière de la Colombie-Britannique a fait l'objet de 2 ans d'étude. Les estimations de la masse sèche sans cendre de feuilles tombées variaient de 201 à 481 g·m^{-2}·an^{-1}. L'importance de l'apport en feuilles caduques était semblable entre les cours d'eau. Par contre, l'apport d'aiguilles de conifères dans le cours d'eau baignant un peuplement mûr était plus important que dans les deux cours d'eau qui traversaient des peuplements de seconde venue. Le facteur de la variation saisonnière de la biomasse de feuilles caduques était supérieur à 60, mais la biomasse d'origine ligneuse et la matière organique totale sous forme de particules grossières montraient moins de variation saisonnière. La décomposition de feuilles d'aulne dans deux des cours d'eau était fortement liée à la température; elle variait nettement d'un cours d'eau à l'autre, celui en retenant la plus grande masse montrant le plus faible taux de décomposition. À partir d'estimations de l'apport et des taux de décomposition, l'auteur a élaboré des modèles de la chute des feuilles pour prédire la biomasse benthique de la couche de feuilles mortes. Des comparaisons des prévisions tirées des modèles et des mesures réelles ont révélé que de 70 à 94 % de la matière végétale était ignorée et probablement éliminée du segment étudié à cause du transport hors du système, du dépôt dans le lit d'inondation ou de l'enfouissement. Les variations saisonnières de la biomasse de la matière organique sous forme de particules grossières font ressortir la variation de l'apport alimentaire dont disposent potentiellement les détritivores lotiques.

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Small streams receive most of their energy inputs from allochthonous materials mostly in the form of detritus from riparian vegetation (reviewed by Anderson and Sedell 1979; Cummins et al. 1983). Some estimates indicate that greater than 60% of the energy of stream community metabolism is contributed by allochthonous detritus (Nelson and Scott 1962; Minshall 1967; Fisher and Likens 1973; Triska et al. 1982; Connors and Naiman 1984). The importance of this material to biological processes in streams makes it vital to understand the pathways and rates of both input and decomposition of detritus. The seasonal timing of inputs and outputs of detrital materials is thought to be especially important to the life cycle phenology (Petersen and Cummins 1974) and population dynamics (Richardson 1991; Richardson and Neill 1981) of species which consume detritus, particularly those species which are obligate detritivores such as shredders.

There are many components to stream detrital budgets and most are difficult to measure directly (Cummins et al. 1983; Webster et al. 1990). It is difficult to directly estimate rates of export of detrital materials, since most of them are transported during high-flow events and rates are dependent on the amount of benthic organic matter (Cuffney and Wallace 1989; Webster et al. 1990). Most previous attempts to sample detritus in transport have been made in first-order streams (for an exception, see Triska et al. 1982) where the entire discharge could be sampled and watershed-wide inputs estimated. Likewise, buried organic matter has also confounded attempts to calculate actual storage (Webster et al. 1990). Detritus budgets have been calculated by measuring several components directly and using indirect estimates of others (Boling et al. 1975; Wallace et al. 1982; Webster 1983; Petersen et al. 1989; Cuffney et al. 1990; Webster et al. 1990). The reported relative values of components differ widely between studies. Part of this variation may be real differences among streams in terms of geography, size,

[1]Present address: Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada V5A 1S6.

and local geomorphology and part may be due to the range of techniques used. Resolution of these issues will require testing different approaches of calculating organic matter budgets.

Different types of detritus have very different nutritional value for consumer species and should be considered separately, but this is rarely done (Bärlocher and Kendrick 1973; Sweeney and Vannote 1986). Conifer needles and woody material decay at very low rates (Sedell et al. 1975; Anderson et al. 1978; Melillo et al. 1983) and apparently play a limited role in the nutrition of stream detritivores (Anderson et al. 1978; Webster and Benfield 1986) in contrast with deciduous leaf material. The timing and rate of detrital input differs depending on the source species. The size and structure of detritus can also affect other stream processes, e.g. large wood debris contributes to detrital storage and substrate stabilization (Triska and Cromack 1980).

In this paper I describe aspects of the dynamics of coarse particulate organic matter (CPOM, detritus $\geq$1.0 mm in diameter) in three small, coastal montane streams of southwestern British Columbia. I have focussed on CPOM for two reasons. First, while CPOM dynamics are complex (Cummins et al. 1983), they are much clearer than fine particulate organic matter (FPOM) dynamics. Second and most importantly, coarse detritus constitutes the food supply for a number of species of aquatic invertebrates, and many detritivores feed on coarse detritus exclusively (or almost so) for much of their larval life. Since detritus type has a large influence on the rate of consumption and growth to detritivores, I have considered major categories of litter separately. I have measured several components of CPOM inputs and outputs in order to estimate budgets for these streams and to compare the potential contribution of export to CPOM dynamics of west coast streams.

Methods and Materials

Study Site

Three second-order streams in the Malcolm Knapp Research Forest of the University of British Columbia (UBC) were studied from 1984 to 1986. The Malcolm Knapp Research Forest is located in the Coast Range mountains approximately 60 km east of Vancouver (122°34′W, 49°16′N). The watersheds lie in the Coastal Western Hemlock forest, and the conifers *Tsuga heterophylla* (western hemlock), *Thuja plicata* (western red cedar), and *Pseudotsuga menziesii* (Douglas fir) are the dominant forest species. The major riparian species are *Alnus rubra* (red alder), *Acer circinatum* (vine maple), and *Rubus spectabilis* (salmonberry), based on litterfall estimates. All three streams drain thin soils of glacial origin, overlying a predominantly acid igneous bedrock (Feller and Kimmins 1979).

These streams were chosen as representative of small streams in the area and also for ease of access. Some of the essential features of the three streams are outlined in Table 1. Blaney Creek is the only stream of the three with a lake upstream of the sampling reach; Placid Lake is about 2 km upstream. Mayfly Creek drains the west side of Mt. Blanchard and has a steeper slope than the other two streams. Spring and Blaney creeks drain more gently sloping parts of the forest (see Dudgeon and Richardson 1988 for a map of the area).

The substrate in all three streams was similar, made up predominantly of cobble, gravel, and sand. Boulders were common in sections of Mayfly Creek but were infrequent in the study section of Blaney Creek and were lacking in the area of study in Spring Creek.

The forest in each drainage basin has had a diverse history of logging and fire. The forest age given in Table 1 is that of the riparian vegetation surrounding the sampling reach. Mayfly Creek drainage was characterized by second-growth forest of western hemlock and cedar. The watershed was logged early this century, and consequently the forest in the Mayfly Creek basin is relatively even aged. Portions of the watershed of Blaney Creek have been logged at different times since 1958, but most of the forest close to the stream was logged and replanted in 1958. The riparian zone around Spring Crreek is old-growth forest, but the majority of the watershed has been logged over the past 110 yr. Some of the watershed was logged for experiments on stream hydrology and forest nutrient budgets upstream of the study reach (Feller 1977; Feller and Kimmins 1979).

Details of water chemistry and underlying geology for similar streams in the UBC Research Forest have been published previously (Feller 1977; Feller and Kimmins 1979). The concentrations of most major ions vary with season and discharge. Average annual pH is 6.8, with electrical conductivity of 20.00 $\mu mho \cdot cm^{-1}$ at 25°C. The average annual concentration of PO_4 was 10 $\mu g \cdot L^{-1}$, nitrate 230 $\mu g \cdot L^{-1}$, and Ca 1.5 $mg \cdot L^{-1}$ (Feller 1977; Feller and Kimmins 1979). Mayfly and Spring creeks are generally shaded by dense riparian vegetation so that stream temperatures vary at most 3°C daily. The seasonal extremes of temperatures recorded from 1983 to 1988 (by maximum–minimum thermometers) were 17.5°C to below 0°C for Mayfly Creek, 17.5–1°C in Spring Creek, and 26–0°C in Blaney Creek. Discharge varies considerably seasonally and is characterized by high and variable winter flows and lower, more stable discharge in summer and early autumn (Feller and Kimmins 1979).

Direct Inputs

Litterfall data were collected using basket traps of aluminum screening (mesh size 1.4 mm) with walls about 4 cm high and a bottom area of 975 cm^2. Ten of these traps were placed at approximately 10-m intervals along the sides of each of the three streams. The traps were placed on boulders or logs within the bankfull perimeter of the stream to sample litter that would

TABLE 1. Summary of stream characteristics at the three study sites. Slope ($m \cdot m^{-1}$) estimates are shown for a 1-km length near the study site.

Stream	Drainage area (km^2)	Approximate slope	Slope in sample reach	Forest age (yr)	Altitude (m a.s.l.)
Blaney	2.9	0.054	0.025	30	380
Mayfly	3.6	0.080	0.038	60	315
Spring	3.8	0.037	0.024	250+	135

fall directly into the streams or be entrained by wind movement or by the flow as discharge increased. Traps were occasionally disturbed or washed away, and these were replaced on subsequent visits. Litter traps were emptied at irregular intervals, and samples were taken more frequently as rates of deposition increased. Litterfall samples were collected from 31 July 1984 to 3 August 1986.

In the laboratory, samples were separated into the following groups: (i) red alder (*Alnus rubra*), (ii) vine maple (*Acer circinatum*), (iii) *Rubus* spp. (*R. spectabilis* and *R. ursinus*), (iv) willow (*Salix* spp.), (v) other deciduous leaves, which included sitka alder (*Alnus sinuata*), dogwood (*Cornus nuttallii*), and huckleberry (*Vaccinium* spp.), (vi) cedar leaves (*Thuja plicata*), (vii) hemlock (*Pseudotsuga heterophylla*) and Douglas fir (*Pseudotsuga menziesii*) needles, (viii) wood, and (ix) miscellaneous plant material such as flower parts, bud scales, lichens, and moss. Each component was dried for at least 24 h at 60°C, weighed, reduced to ash in a muffle furnace at 500°C, and then reweighed to determine ash-free dry mass (AFDM).

Benthic Detritus Standing Crops

Samples of benthic detritus were collected approximately each month using a cylindrical sampling device of sheet metal with a net on the downstream side with mesh size 102 μm and sampling area of 712 cm^2. After the macroinvertebrates were removed, the remaining material was sorted by sieve, and materials larger than 5 mm in diameter were sorted into deciduous leaves, cedar, hemlock or Douglas fir needles, wood, and a category for miscellaneous detritus. Detritus 1–5 mm in diameter was treated as one category. All detritus from benthic samples was dried, weighed, ashed, and expressed as AFDM.

Breakdown Rates

Decay rates of plant detritus were estimated using leaf packs of red alder. The leaves for this study were collected shortly after abscission, air-dried, heat-sealed in plastic bags, and stored in a deep-freeze (−20°C) until needed. The leaves were weighed into groups of 5 g (±0.025 g), fastened together using pieces of monofilament nylon, and then loosely tied to concrete bricks as described by Petersen and Cummins (1974). Fifteen to 25 leaf packs were placed in either Mayfly Creek or Spring Creek on different dates and randomly chosen subsets were retrieved on five different dates from each series. The first subset was collected 2 d after placement to serve as an initial weight, controlling for losses due to handling and leaching, the latter of which results in rapid loss of mass (Webster and Benfield 1986). In the laboratory, leaves were rinsed twice to remove macroinvertebrates. All leaf fragments >1 mm were added to the sample, which was dried, weighed, ashed, and reweighed as described above. These data produced one decay rate curve for each series of leaf packs. The only exception to this protocol was for the 1987 series where leaf packs were placed in the stream on five different occasions and retrieved simultaneously 2 d after the last set was placed (J. S. Richardson, unpubl. data).

Temperatures were measured with maximum–minimum thermometers (calibrated against a laboratory thermometer). On each visit to the sampling locations, I recorded the temperature at the time of observation and the maximum and minimum temperatures for the period since the previous visit.

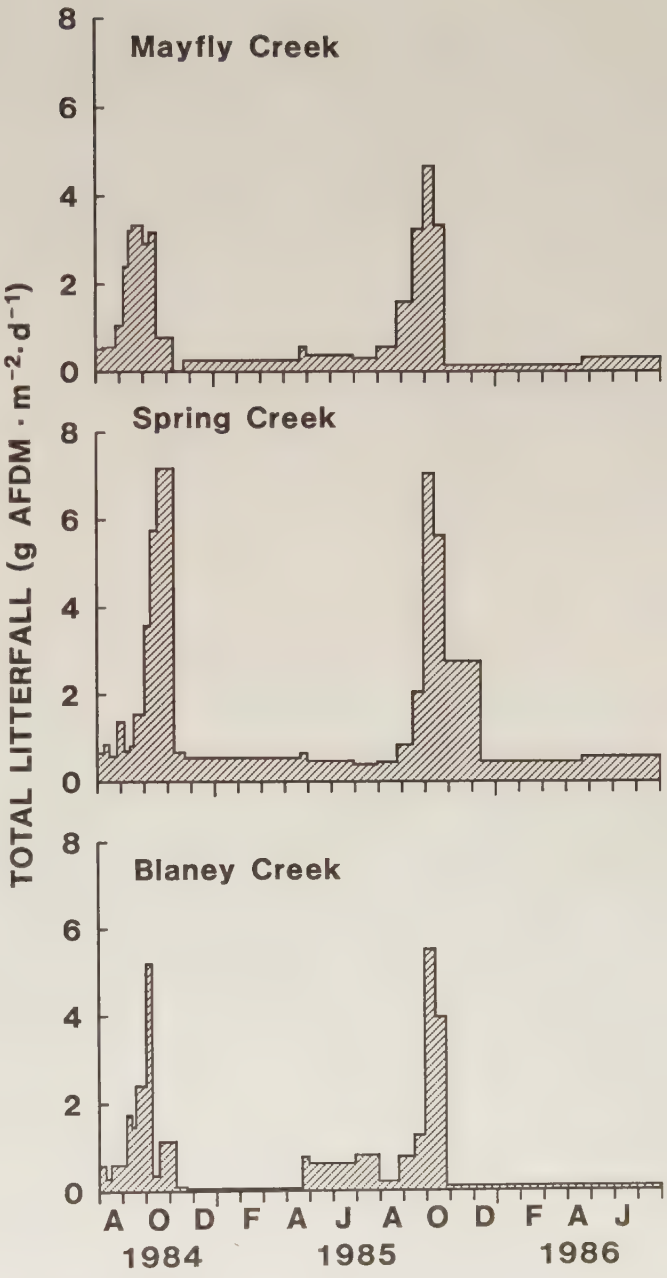

FIG. 1. Daily litterfall rates for all detritus collected in litter traps along the margins of UBC Research Forest streams. The rates are averages for each sampling interval.

Results

Direct Litterfall

The input of all detrital materials was highly seasonal and occurred primarily in the autumn (Fig. 1). During the 2 mo from mid-September to mid-November, more than half the annual litterfall was collected for each stream (56% for Mayfly and Spring creeks and 63% for Blaney Creek). In general there was little direct litterfall from December through April for any of the streams, and rates were low during late spring and summer. In July and August, red alder trees dropped moderate numbers of small, green, and apparently intact leaves in all years from 1983 to 1988 (J. S. Richardson, pers. obs.).

The total amounts of litter input were similar between years within each stream. The input to Spring Creek was highest

TABLE 2. AFDM ($g \cdot m^{-2} \cdot yr^{-1}$) of components of direct litterfall to small coastal streams. Grand totals at the bottom represent annual (July–July) litterfall of all plant detritus. Collection periods were from 31 July 1984 to 30 July 1985 and from 31 July 1985 to 30 July 1986.

	Mayfly Creek		Spring Creek		Blaney Creek	
Component	1984–85	1985–86	1984–85	1985–86	1984–85	1985–86
Red alder	121.6	136.9	122.7	109.3	131.1	123.6
Vine maple	35.7	30.7	27.5	27.0	3.8	1.2
Rubus spp.	15.8	17.3	18.2	11.6	11.5	3.4
Willow	1.7	3.5	0.4	0.1	29.0	10.6
Total deciduous[a]	174.8	193.0	172.2	132.4	150.1	153.4
Cedar	7.2	32.3	114.6	137.3	0.2	0.6
Hemlock and Douglas Fir	2.5	1.4	49.0	65.2	2.5	18.7
Total coniferous	9.7	33.7	163.6	202.5	2.7	19.3
Wood	48.1	16.5	32.6	48.8	36.9	10.6
Totals[b]	248.8	261.7	448.5	481.5	219.7	201.8

[a]Total deciduous leaves also includes leaves of species not listed explicitly.
[b]Grand totals include miscellaneous plant parts.

followed by Mayfly and Blaney creeks (Table 2; Fig. 1). The total fall of detritus into Spring Creek was more than double that of Blaney Creek and about 1.8 times that of Mayfly Creek. The input of deciduous leaves was similar among streams with annual inputs of 132–193 $g \cdot m^{-2} \cdot yr^{-1}$ (Table 2). Mayfly Creek had the highest relative input of deciduous leaves of the three streams, accounting for 70 and 74% of the total input for the 2 yr respectively. Red alder was the most abundant source of deciduous litter, accounting for over 69% of deciduous leaf AFDM collected in the litter traps. The main difference between the three streams was in the amount of input from coniferous species. Spring Creek received 6.9 times the coniferous input of Mayfly Creek and 16.7 times that of Blaney Creek (Table 2).

In all three streams the input of deciduous leaves was highly pulsed (Fig. 2), with most leaves entering from mid-September to the end of November. The timing of conifer leaf fall was also primarily autumnal, especially for red cedar (Fig. 3). Hemlock and Douglas fir leaves (which were not separated, but were mostly hemlock) fell slightly more often during the autumn but continued to drop leaves throughout the year.

Benthic Detritus

Woody material ≥5.0 mm in diameter made up the largest portion of CPOM standing crop in Mayfly Creek during 1985–86 (Fig. 4). Particles ≥5 mm from deciduous leaves were much less abundant than wood. CPOM in the particle size of 1.0–5.0 mm was also abundant, but the nature of these particles was not assessed. However, the representation in each category did not appear to be proportional to the amounts of wood, deciduous leaves, or conifer leaves ≥5.0 mm.

The standing crop of deciduous leaf material showed the seasonal pattern anticipated, with higher densities during late autumn and winter than the remainder of the year (Fig. 4 and 5). There was a 63-fold difference from the peak during January to the minimum of April for Mayfly Creek deciduous material and a 62-fold difference for Spring Creek. Abundance of leaf particles during summer was slightly higher than spring values. Standing crops of wood (≥5 mm) and total CPOM were variable over the year and exhibited seasonal patterns with low summer abundance and higher standing crop during the remainder of the year. Estimates of annual average standing crops of CPOM were approximately 32 $g \cdot m^{-2}$ for both Mayfly and Spring creeks. Standing stocks for leaf litter >5.0 mm in diameter were only 2.8 $g \cdot m^{-2}$ for Mayfly Creek and 4.7 $g \cdot m^{-2}$ for Spring Creek.

The spatial distribution of detrital materials ≥1 mm was heterogeneous, which is reflected in the large standard errors for the estimates of abundance. The patchiness of leaf detritus distribution was more obvious during winter whereas particle sizes were smaller and accumulations lower during spring and summer. Woody materials were always very patchily distributed (Fig. 4 and 5).

Leaf Pack Breakdown Rates

Nineteen separate series of decomposition trials were run in either Mayfly Creek or Spring Creek (Table 3). Leaching and handling losses were from 8 to 17% of the AFDM over 2 d. Linear and negative exponential (natural logarithm transformed data) models were fitted to the data in an analysis of covariance (ANCOVA) with each trial nested within stream (Proc GLM, SAS Institute Inc. 1985). Both analyses produced similar results; however, the linear model explained about 80% of the total variance whereas the negative exponential accounted for less than 60% of the variance. Accumulated degree-days (above 0°C) was entered as the covariate in the ANCOVA and accounted for the largest portion of the total variance at >65%. There was a significant difference between streams in the rate of decay corrected for temperature as shown in the stream by degree-day interaction (ANCOVA, $P < 0.002$). Decay rates in Mayfly Creek were significantly higher compared with Spring Creek when the slopes of the leaf remaining versus degree-day regressions were compared (Mayfly: $a = -0.0043 \pm 0.00018$ SE, $n = 289$; Spring: $a = -0.0039 \pm 0.00017$, $n = 100$). The calculated exponents (negative exponential model) of the decay curves were −0.00206 and −0.00165 for Mayfly Creek and Spring Creek, respectively.

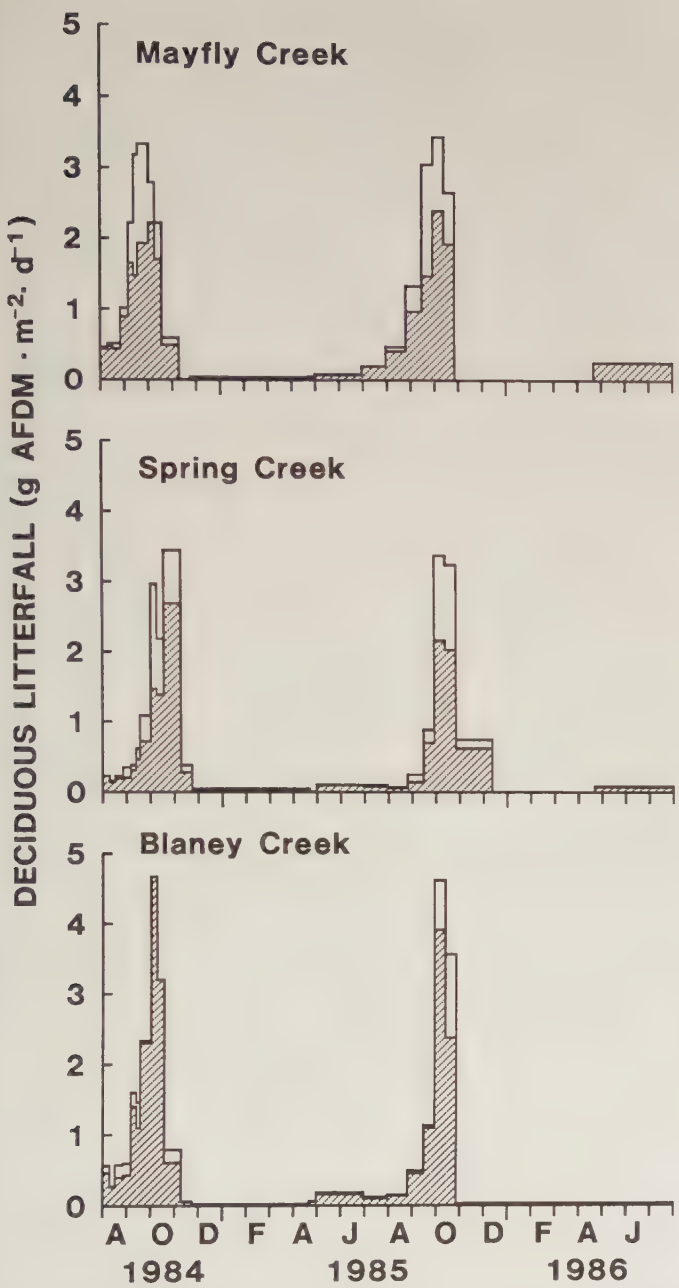

FIG. 2. Daily litterfall rates for deciduous leaves collected in litter traps along the margins of UBC Research Forest streams. Shading indicates the contribution of red alder. The rates are averages for each sampling interval.

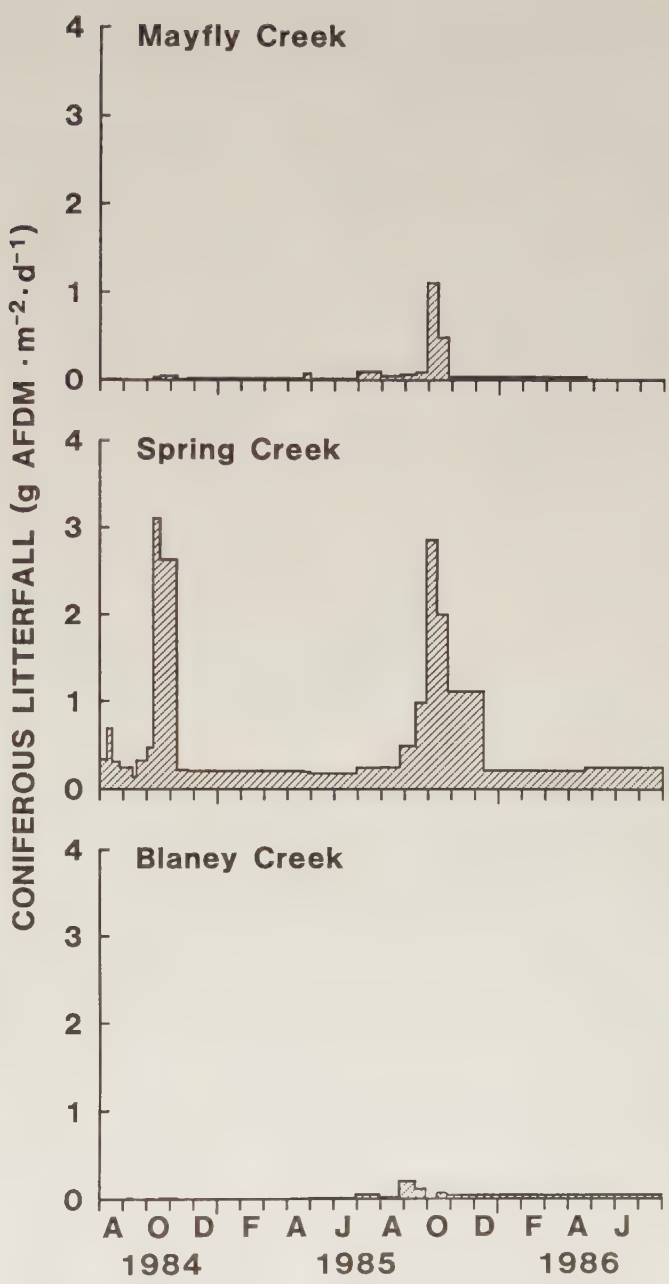

FIG. 3. Daily litterfall rates for coniferous leaves collected in litter traps along the margins of UBC Research Forest streams. The rates are averages for each sampling interval.

To determine the temperature effect on decay rate, the rate of leaf loss per day for each trial (the slope of the linear regression) was regressed against the average temperature during the incubation period. There was a significant exponential increase in the rate of decomposition as temperature increased (Fig. 6, $R^2 = 0.41$, $P < 0.002$). The relationship was not strongly curvilinear, since a linear relationship fit nearly as well ($R^2 = 0.31$, $P < 0.01$).

The 1987 decomposition trial compared the rate of decay of alder leaf packs in riffles versus pools of Mayfly Creek. The data for dry mass remaining were fitted to an ANCOVA with the number of days in the stream as the covariate. The number of days in the stream was the only significant variable (ANCOVA, $F_{1,51} = 617.5$, $P < 0.0001$). A linear model gave a better fit to the data ($R^2 = 0.92$) than a negative exponential model ($R^2 = 0.66$). The ANCOVA showed that there were no significant differences in decomposition rates between riffles and pools or between upstream or downstream sections of the stream (all with $P > 0.17$, Fig. 7), as indicated by the slopes of the lines. The slopes of the decay curves were similar in the riffle and pool habitats, i.e. 0.081 and 0.068 versus 0.057 and 0.079 for riffles and pools, respectively. The exponent of the negative exponential decay curve was -0.047.

The Model

A simple model combining estimates of deciduous leaf input and temperature dependent decomposition rates was used to predict the amount of leaf litter on the stream bottom. I used

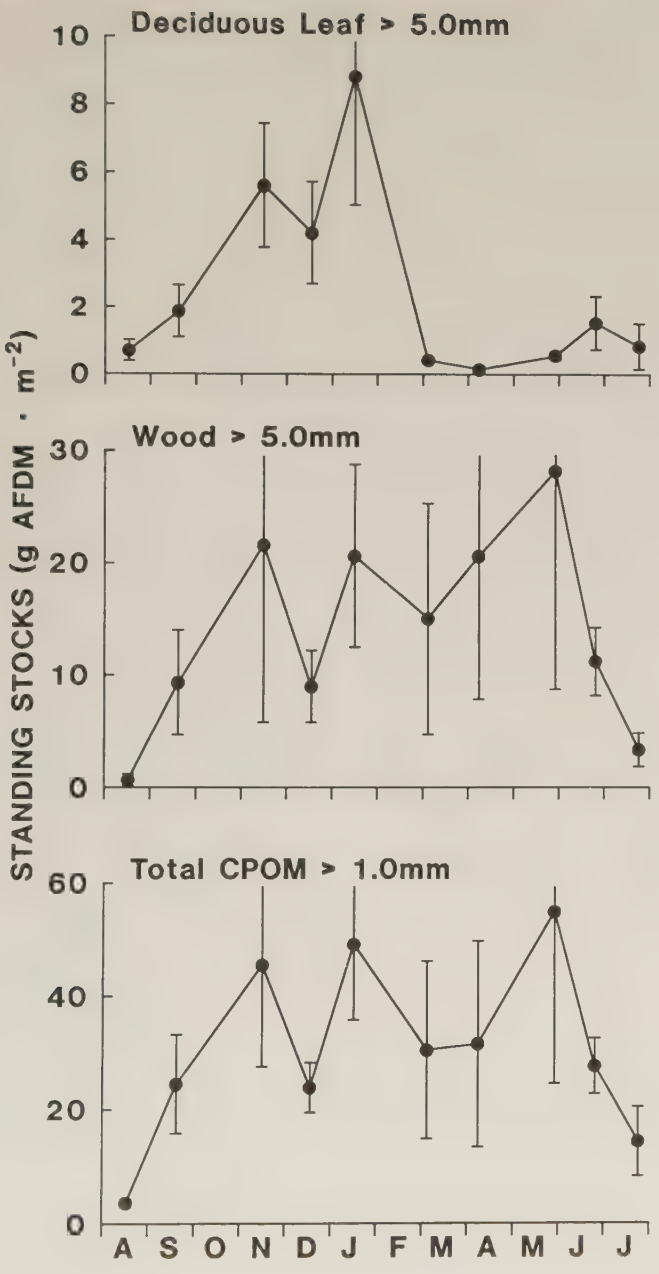

FIG. 4. Estimates (±1 SE) from Mayfly Creek of benthic standing crops from August 1985 to July 1986 of deciduous leaf detritus ≥5.0 mm in diameter, wood detritus ≥5.0 mm in diameter, and all detritus ≥1.0 mm in diameter. For each estimate, sample size was ≥5. Note the different scales on the ordinates.

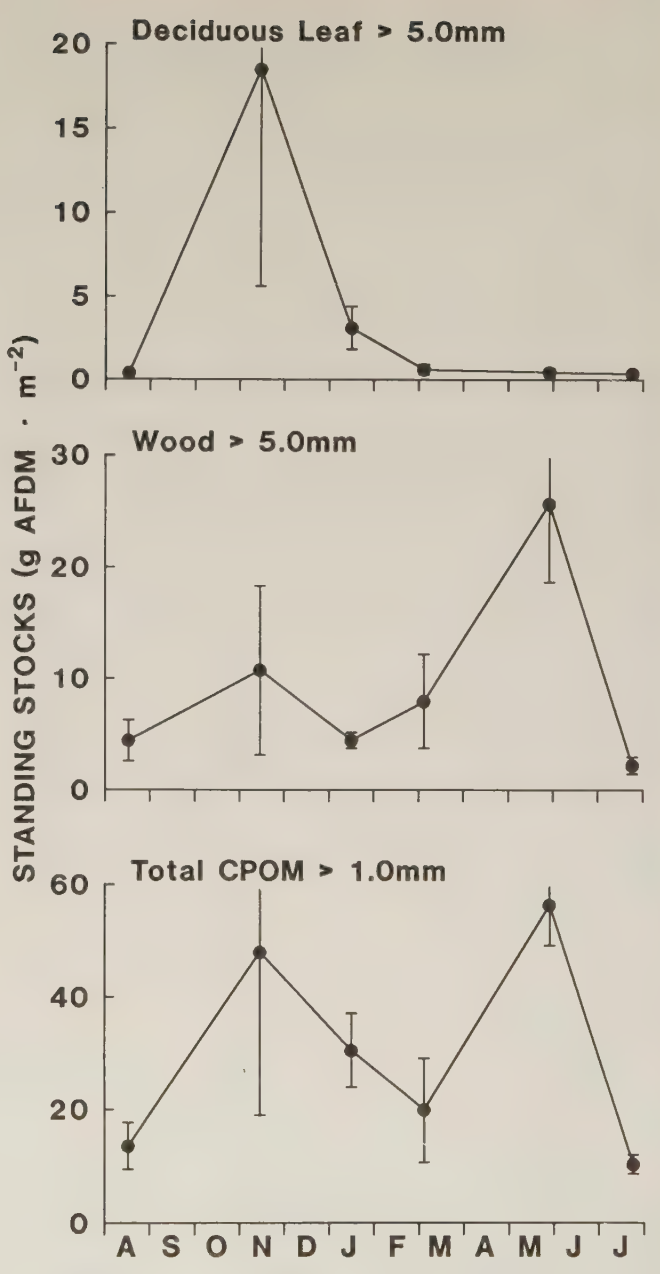

FIG. 5. Estimates (±1 SE) from Spring Creek of benthic standing crops from August 1985 to July 1986. Symbols and categories as for Fig. 4. Sample size for each date was 4.

the decay rates for alder leaf packs, since alder was the most common deciduous input to these streams and since leaves of vine maple and *Rubus* spp. also have rapid decay rates. Two forms of the model, one using a linear decay rate and the other using the negative exponential relationship, were used to make predictions of benthic detritus standing crops. The rates determined for each stream were used in the models.

The results of the simulations were very different, with the negative exponential model predicting much higher standing stocks than the linear decay rate model (Fig. 8). The predictions of mean annual standing stocks were only 38.8 g·m^{-2} (negative exponential model) and 16.3 g·m^{-2} (linear model) for Spring Creek. The predictions for Mayfly Creek were 44.2 and 20.1 g·m^{-2} for the exponential and linear models, respectively. The direct estimates of the benthic standing stock of deciduous leaves >5.0 mm were 29 and 12% of the predictions of the linear and exponential models, respectively, for Spring Creek. The estimates from Mayfly Creek were 14 and 6% of the linear and exponential model predictions, respectively. Based on the proportion of deciduous leaf biomass of all CPOM >5 mm (14% for Spring Creek and 21% for Mayfly Creek), I estimated the amount of leaf tissue likely to be in the 1–5 mm size fraction as 0.11 and 0.2 g·m^{-2} for Spring Creek and Mayfly Creek, respectively. This was likely an overestimate. The addition of leaf matter in the smaller size fraction increased observed benthic leaf matter to 30 and 12.4% of that predicted for Spring Creek and 15 and 6.8% of the prediction for Mayfly Creek.

TABLE 3. Summary of the alder leaf pack decomposition trials. The initial number of leaf packs is indicated in parentheses after the number recovered. The exponents are for the negative exponential decay curve of leaf decomposition rate.

Trial	Stream	Dates	Number recovered	Exponent	Average temperature (°C)
1	Spring	3 June to 3 Aug. 1985	25 (25)	−0.027	13.2
2	Mayfly	3 June to 3 Aug. 1985	22 (25)	−0.040	12.3
3	Spring	3 July to 3 Sept. 1985	25 (25)	−0.023	13.7
4	Mayfly	3 July to 3 Sept. 1985	23 (25)	−0.023	13.0
5	Spring	5 Sept. to 14 Nov. 1985	21 (25)	−0.006	8.7
6	Mayfly	5 Sept. to 15 Nov. 1985	20 (25)	−0.007	6.8
7	Spring	5 Oct. to 14 Nov. 1985	15 (15)	−0.004	7.4
8	Mayfly	5 Oct. to 15 Nov. 1985	15 (15)	−0.004	4.9
9	Mayfly	1 May to 26 June 1986	14 (15)	−0.011	8.8
10	Spring	1 May to 26 June 1986	15 (15)	−0.011	10.6
11	Mayfly	15 May to 10 July 1986	15 (15)	−0.010	9.8
12	Mayfly	29 May to 24 July 1986	15 (15)	−0.011	10.7
13	Mayfly	12 June to 7 Aug. 1986	15 (15)	−0.019	11.2
14	Mayfly	26 June to 21 Aug. 1986	14 (15)	−0.010	11.8
15	Mayfly	10 July to 4 Sept. 1986	15 (15)	−0.026	12.4
16	Mayfly	7 Aug. to 2 Oct. 1986	15 (15)	−0.027	12.6
17	Mayfly	18 Sept. to 14 Nov. 1986	15 (15)	−0.013	7.1
18	Mayfly	14 Nov. 1986 to 24 Jan. 1987	18 (18)	−0.012	2.0
19	Mayfly	27 May to 16 July 1987	57 (60)	−0.047	11.2

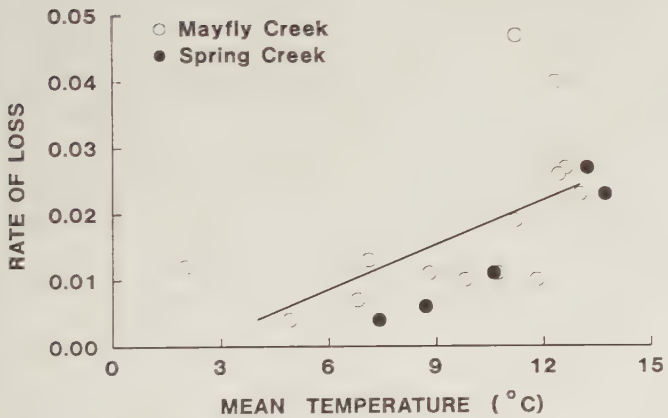

FIG. 6. Relationship between rate of loss per day ($g \cdot g$ dry $mass^{-1} \cdot d^{-1}$) for 5-g leaf packs of red alder versus the average temperature during the incubation period. Values on the ordinate are the exponents from the negative exponential regressions given in Table 3.

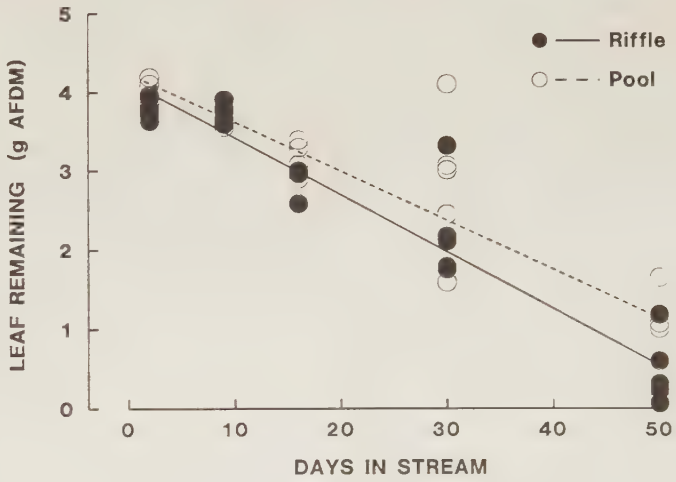

FIG. 7. Comparison of the decomposition rates of red alder leaf packs in riffles and pools of Mayfly Creek. Points represent mass of leaf pack remaining after different lengths of time in the stream. The number of leaf packs recovered was 57; some points are hidden.

Discussion

The standing crop of benthic organic matter only roughly reflected the patterns of litter inputs and decomposition. The major differences between this study and others were quantitative differences in processing rates and benthic standing stocks. Amounts of benthic CPOM in these coastal streams were very low and most of the loss was unaccounted for by estimates of breakdown rates of experimental leaf packs. The apparent differences among streams in terms of seasonal variations of CPOM standing stocks set the stage for important constraints on the biology of consumer species (Richardson 1991; Richardson and Neill 1991).

The litterfall data indicate annual input typical of the published ranges (Anderson and Sedell 1979; Conners and Naiman 1984). Estimates of litter input for small streams vary from about 200 to almost 800 $g \cdot m^{-2} \cdot yr^{-1}$ (Connors and Naiman 1984; Webster et al. 1990). The values I obtained for annual litterfall fit within this range. I did not measure the contribution of blow-in to allochthonous inputs. Blow-in typically contributes 14–25% of total litter inputs per unit area for small streams; however, some estimates have reported as much as half of total input from this source (Triska et al. 1982; Connors and Naiman 1984; Naiman et al. 1987; Webster et al. 1990). If blow-in contributes a similar proportion of litter input to these British Columbian streams, then input rates are still typical of small streams.

The litterfall differences between my study streams and the relative constancy within the streams between years were related to differences in the canopy. Spring Creek was the only stream that I studied with a completely closed, coniferous canopy of western hemlock and red cedar. The riparian forest along Spring Creek is about 250+ yr old and the major difference in litterfall rates between Spring Creek and the other two streams

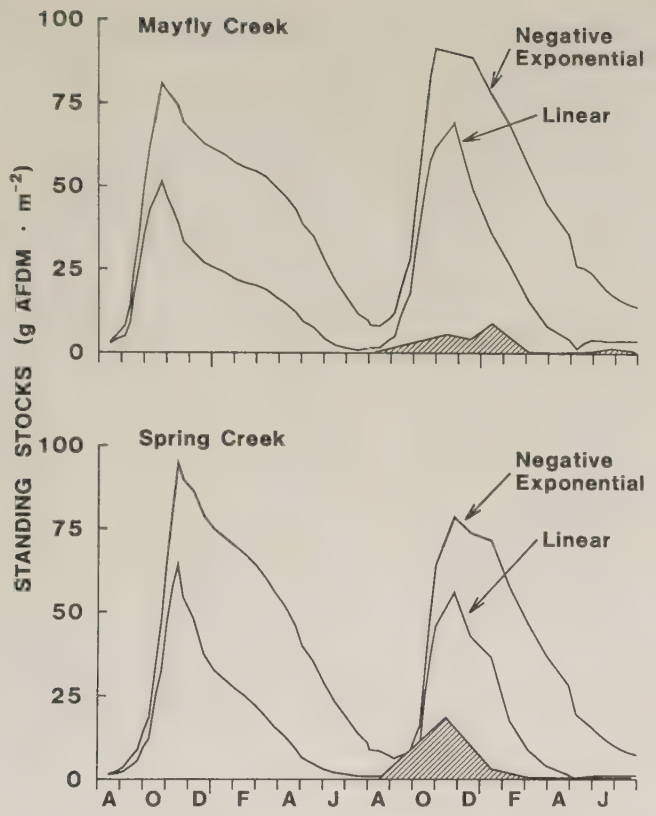

FIG. 8. Predicted benthic standing crops of deciduous leaf litter based on a computer model incorporating litterfall estimates and two versions of the temperature-dependent decay functions for each stream. The lines indicate the predictions using a negative exponential decay function and a linear decay model. The shaded region represents the direct estimates of standing stock of leaf tissue >5.0 mm for the period August 1985 to July 1986 (from Fig. 4 and 5).

was in the amount of coniferous leaf input. While Mayfly Creek is surrounded by coniferous forest of 60 yr of age, the reach I studied had active banks with dense growths of deciduous trees such as alder and vine maple on the disturbed patches. The young age of the coniferous trees surrounding Blaney Creek accounts for the low rate of conifer needle inputs to this stream.

While input rates of deciduous leaf detritus to these montane streams were low during summer, there was some direct litterfall of green leaves of alder. Entry of green leaves to streams has been found to result from beaver activity (Stout et al. 1985) or severe storms (McArthur et al. 1986). Red alders appear to drop small numbers of leaves during July, August, and September in British Columbia. This pattern of summer leaf drop was observed each year from 1983 to 1988 (J. S. Richardson, pers. obs.). The addition of small amounts of leaf material during summer is likely to be important to the energetic subsidy of detritivore populations, given evidence that these animals are food limited (Richardson 1991).

There were large differences between the composition of litterfall and benthic detritus. Deciduous leaves accounted for 72% of annual litterfall to Mayfly Creek; however, they represented only 8% of the mean annual benthic standing crop of CPOM. On the other hand, wood only made up 12.7% of the measured litterfall whereas it comprised 46% of CPOM in the stream bottom. My estimates of wood input are surely underestimates, since most wood enters as branches and boles ≥15 cm in diameter so that small litter traps are inappropriate to the spatial and temporal scales of wood input. In addition to this sampling problem, wood decomposes at an exceedingly low rate in comparison with leaves (Anderson et al. 1978; Anderson and Sedell 1979; Webster and Benfield 1986), so that there is a large amount of between-year storage, and fragments from large bole wood can be generated for many years (Triska et al. 1982; Ward and Aumen 1986). Estimates of benthic detritus from other small, coniferous forest streams indicate that >90% of standing crop is wood (Naiman and Sedell 1979; Triska and Cromack 1980).

Estimates of average annual standing crops of CPOM were low in comparison with many other studies. CPOM standing stocks for some streams in eastern North America ranged between 100 and 250 $g \cdot m^{-2}$ (Golladay et al. 1989; Petersen et al. 1989), more than an order of magnitude higher than those reported here. Estimates of benthic CPOM from a steep Rocky Mountain stream (Short and Ward 1981) were similar to those measured for Mayfly and Spring creeks. Deciduous leaf material represents a small and variable portion of benthic CPOM, but it is still the most important component of detritus as a food source (Cummins et al. 1989). The estimates of standing crops of leaf detritus in Mayfly Creek were low in comparison with values from other small streams. Few species of stream macroinvertebrates derive a large portion of their nutrition from decaying wood (Anderson et al. 1978) in spite of comprising a larger portion of benthic organic matter.

Decomposition rates of red alder leaves increased as temperature increased. This temperature dependence has been reported previously (Peterson and Cummins 1974; Hanson et al. 1984; McArthur et al. 1988; Short and Smith 1989). The important aspect of temperature-dependent rates of decay is that CPOM is decomposed faster during the time of year when it is least abundant, exacerbating the seasonal shortage of food for detritivorous species (Richardson 1991).

There were significant differences in decomposition rates of leaves in the two streams after accounting for differences in accumulated degree-days. Part of this difference might be due to the slight differences in retentiveness in the streams, since Spring Creek has a slightly higher annual standing crop, which might provide greater food base to consumers. However, there are other differences between the streams that might affect decomposition rates, and the rates were not vastly dissimilar.

Loss rates of alder leaf packs did not differ significantly between riffles or pools. However, there were much higher densities of macroinvertebrates on leaf packs in riffles (J. S. Richardson, unpubl. data). This suggests that microbial contributions to processing may compensate in the case of reduced macroinvertebrate densities, as estimates of the rates of leaf conversion in a Michigan stream indicated that microbes consumed 13 times the amount that animals did (Petersen et al. 1989). If the leaves had been in natural accumulations in pools, rather than attached to bricks and exposed to water flow, decomposition of most of the leaves would likely have been anaerobic.

There are many difficulties associated with measurements of export rates of detritus from streams. Among the problems include the technical difficulty of measuring transport during high-flow events when most export of organic matters occurs (Cuffney and Wallace 1989; Webster et al. 1990). The two main approaches to estimating loss of detritus by export are (1) direct measurement of transport and (2) accounting for differences in benthic standing stocks due to biological conversion; the remainder is assumed to be lost to export (Cuffney

et al. 1990). The difference method is the one on which my model is based. There are reasons to believe that the model I have presented underestimates the true losses of leaf tissue due to export. First, the decomposition rates determined from leaf pack experiments do not take into account losses due to fragmentation, i.e. lost from the leaf pack but not from the stream, and therefore overestimate loss rates. This would result in underestimates by the model. Second, I did not factor input due to blow-in into the model, and therefore, real export may have been up to 25% higher than calculated. Third, the leaf decomposition rates reported here may also have been overestimated by using dried leaves, since recently fallen leaves may decompose more slowly than previously dried leaves (Gessner and Schwoerbel 1989). Webster (1983) used a difference model to predict CPOM dynamics and found a large discrepancy between observed and predicted values which he attributed to errors in parameter estimation. On the other hand, Boling et al. (1975) found that a similar model accurately predicted standing stocks of detritus in a Michigan stream. These discrepancies between applications of similar models may reflect real differences in the importance of losses to transport between stream systems. Errors in estimation are inherent to both methods of export calculation. However, it is likely that the true value of leaf loss from British Columbian streams due to export and floodplain deposition is greater than the 71–94% I have estimated for leaf fragments >5 mm or the 70–93% export predicted for all leaf fragments >1 mm. The material lost locally to export may be flushed to areas far from the entry point, such as downstream lakes or large pools, or lost to floodplains or nearby pools.

The estimates of losses due to export for the streams I studied are at the high end of previously published values. The few published values for export range from 2 to almost 100% of total inputs (Cummins et al. 1983; Cuffney et al. 1990; Webster et al. 1990). Direct measures of export rates suffer from technical problems, as previously mentioned, but also do not usually account for redeposition from transport (for an exception, see Webster 1983). Another source of error in direct measures of hydrologic transport of CPOM is in estimating the watershed inputs of litter to streams and the assumption that input rates are uniform throughout the watershed. All attempts at calculating budgets for streams face the difficulty of buried organic matter, but use the simplifying assumption that surface and buried CPOM is in long-term equilibrium (Cummins et al. 1983; Webster et al. 1990).

There are several features that may affect retention of CPOM, including seasonality and magnitude of discharge variation, and geomorphology, for instance slope and substrate type. Winter hydrographs for west coast streams are extremely variable and freshets occur at a time when most of the litter is arriving in the stream. This timing may account for much of the loss that takes place. The extent to which each of these factors influences stream budgets has not been evaluated. Ross (1963) noted that there was a concordance between communities of shredders in streams and the forest types through which the stream flowed. It is possible that the instream processes that affect the seasonality and magnitude of CPOM standing crop are also correlated with forest types through geographic location and that instream processes may have an important effect on benthic communities beside forest type per se.

These results demonstrate some of the patterns of litter entry and decomposition that contribute to seasonal changes in the abundance of coarse detrital materials. There are many other aspects to the dynamics of detritus, such as quality changes, burial (Cummins et al. 1983), and heterogeneity of distribution within and between reach types (Lamberti et al. 1989). The three primary sources of seasonal variation in detrital standing crop are timing of litterfall, temperature effects on decomposition rates, and winter and spring flushing associated with increased discharge. The variation in abundance of assimilable detritus sets the pattern of resource availability to detritivores. The seasonal variation in detrital standing crops in streams creates population bottlenecks in terms of growth and survivorship of shredders (Richardson 1991). Research dealing with the seasonal dynamics of detritus should focus on temporal patterns of resource availability to consumers.

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In vitro Cultivation of *Contracaecum osculatum* (Nematoda:Anisakidae) from Third-Stage Larvae to Egg-Laying Adults

C. G. Likely and M. D. B. Burt

Department of Biology, University of New Brunswick, Fredericton, N.B. E3B 6E1, Canada

Likely, C. G., and M. D. B. Burt. 1992. In vitro cultivation of *Contracaecum osculatum* (Nematoda:Anisakidae) from third-stage larvae to egg-laying adults. Can. J. Fish. Aquat. Sci. 49: 347–348.

Third-stage (L_3) *Contracaecum* sp. larvae, from *Gadus morhua*, developed to sexual maturity in a two-step roller culture system using medium API-1 supplemented with 5 mM L-cysteine only (step 1) and with bovine haem (Fildes' reagent) added (step 2). The gas phase was 85% N_2 – 10% CO_2 – 5% O_2 and the culture was maintained at 35°C. The mature nematodes proved to be *Contracaecum osculatum*, a species which normally matures in the stomach of pinnipeds.

Le plein développement sexuel du troisième stade larvaire de *Contracaecum* sp., parasite de *Gadus morhua*, a été obtenu selon la technique de culture sur rouleaux en deux étapes à une température de 35°C. Le milieu de culture choisi était de l'API-1, substance à laquelle on a ajouté 5 mM de L-cystéine pour la première étape et du hème bovin (réactif de Filde) pour la deuxième étape. La phase gazeuse présentait un rapport de 85% N_2 – 10% CO_2 – 5% O_2. Les nématodes matures se sont révélés être des *Contracaecum osculatum*, une espèce qui atteint généralement la maturité dans l'estomac de pinnipèdes.

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Little is known of the life cycle of *Contracaecum osculatum*, an anisakine nematode which matures in the stomach of pinnipeds and is cosmopolitan in distribution (Davey 1969). Infective (L_3) larvae of *Contracaecum (Phocascaris)* spp. occur in a wide variety of marine fishes, many of which, like the cod (*Gadus morhua*), are commercially important (Wootten 1978; Elarifi 1981). Although usually found in the body cavity, these larvae occur also in the flesh of fish hosts and hence are considered potential human pathogens if eaten alive in raw or inadequately cooked fish (Jackson 1975; Margolis 1977; Smith and Wootten 1984). Unfortunately, *Contracaecum (Phocascaris)* spp. larvae are morphologically difficult to identify to species (Berland 1961). However, larvae encysted on, or in, the liver of Baltic cod (*Gadus morhua*) and larvae on the pyloric caecae of capelin (*Mallotus villosus*), cod, and plaice (*Hippoglossoides platessoides*) have tentatively been identified as *C. osculatum* by Fagerhölm (1988) and McClelland et al. (1985), respectively.

In the present study, we identified *C. osculatum* larvae from the viscera of cod from the Gulf of St. Lawrence by successfully culturing the nematodes in vitro to sexual maturity, thus confirming the identification of McClelland et al. (1985).

Materials and Methods

Contracaecum sp. larvae (8-10 mm long) were recovered from the viscera of cod from western Newfoundland. The larvae were obtained by digestion procedures using 3 g of pepsin and 3 mL of HCl made up to 100 mL with 0.85% saline and held at room temperature for 4 h. After three rinses in sterile 0.85% saline, larvae were washed in 100 mL of Earle's balanced salt solution containing 1 mL of Gibco Antibiotic-Antimycotic (Cat. No. 600-5245SE). The larvae were cultured in a two-step roller culture system according to the procedures of Douvres and Malakatis (1977). Groups of 20 larvae were placed in 500-mL roller bottles with 40 mL of API-1 (supplemented with 5 mM L-cysteine) at pH 4.5 and a 85% N_2 – 10% CO_2 – 5% – O_2 gas phase. On the seventh day of cultivation and every sixth day thereafter, larvae were transferred aseptically to clean sterile bottles, containing 80 mL of API-1 supplemented with 5 mM L-cysteine and 48 μg of bovine haem. Moribund larvae were removed on transfer and fixed in hot 70% ethanol and cleared in lactophenol for measurements. Ova were collected by digesting used medium and washing the digest through a series of stainless steel sieves of 212, 102, 70, and 45 μm, the last two sieves catching the ova.

Results and Discussion

The L_3 larvae from cod possess a pyramidal boring tooth situated between the primordial lateroventral lips close to the slitlike opening of the excretory pore and opposite the large primordial dorsal lip. The nerve ring is located anteriorly about 0.25 mm from the tooth. The excretory duct runs back from the excretory pore and expands into the excretory canal which is a whitish color. The oesophagus comprises a long preventriculus, of approximately 1 mm, and a short bulblike ventriculus 0.05 mm long. The ventriculus bears a ventricular appendix running posteriorly along the intestine as well as an intestinal caecum which runs anteriorly along the preventriculus. Posteriorly, the conical tail tapers steadily and terminates without a mucron or spine. The larvae in fish are generally between 7 and 20 mm and have a luminescent green herringbone pattern along the intestinal tract.

When placed in medium at 35°C, after an initial 3- to 4-h period of vigorous movement, the larvae remained quiescent

TABLE 1. Time (days) of development of three anisakid nematodes in vitro.

Species	3rd moult	4th moult	Young adult	Mature
Anisakis simplex	2–3	15–18	20–30	33–35
Pseudoterranova decipiens	4–6	12–14	17–21	28–35
Contracaecum osculatum	10–17	30–39	32–41	37–46

for 2–3 d before subsequently attaching to the wall of the glass culture vessel by means of a cephalic cap. The moult (M_3) to the fourth larval stage (L_4) was completed after 10–17 d of cultivation. The L_4 larvae have three large cuticular lips, small knoblike interlabia, and a cuticular collar. The increase in length is minimal at this stage.

The final moult (M_4) to the adult required 2–3 d to complete and occurred 30–39 d postincubation. The adults, possessing interlabia, grew rapidly and reached maturity within 7–10 d. Mature females were 50–63 mm long with a didelphic reproductive system similar to that found in other anisakines but without a large-muscle mass anterior to the vulva. Mature males were 42–45 mm long and possess a pair of short, straight, unadorned spicules of unequal length. The cultivated nematodes were similar in size and morphology to *C. osculatum* adults (females: 48–71 mm; males: 36–38 mm) in naturally infected harbour seals (*Phoca vitulina*) off the coast of Scotland. In earlier in vitro studies, McClelland and Ronald (1974) obtained preadults (young adults) 13.1–17.2 mm long, while Fagerhölm (1988) was unable to grow larvae even to the L_4 stage from L_3 in vitro.

The life cycle of *C. osculatum* is not known (Davey 1969; McClelland and Ronald 1974; Fagerhölm 1988). Davey (1969) showed that two species of harpacticoid copepods were susceptible to infection with newly hatched *C. osculatum* (L_2) larvae but the nematodes did not grow in the experimental hosts. Choudhury (1989) successfully infected harpacticoid and calanoid copepods as well as decapods with L_2 larvae of *C. osculatum*, but he was not able to transmit the larve to fish hosts. Elarifi (1981) and Smith et al. (1990) found that L_2 larvae, artificially exsheathed and injected into the body cavity of fish, would develop without passing through a crustacean host.

Sudarikov and Ryzhikov (1951) have suggested that the related anisakid *C. osculatum baicalensis*, which parasitises the freshwater Baikal seal (*Phoca sibirica*), may have as its crustacean intermediate host the amphipod *Macrohectopus branickii*. This crustacean is a major food item to the goby (*Cottocomephorus grewingki*) which generally carries an abundance of larval (L_3) *C. osculatum baicalensis*.

In our in vitro system, it was found that *C. osculatum* grew more slowly than either of the two related anisakines, *Pseudoterranova decipiens* (Likely and Burt 1989) and *Anisakis simplex* (unpublished data). Moreover, M_3, M_4, and maturation occurred only when the quantity of L-cysteine in the medium was increased by 5 mM. Nevertheless, the worms appeared robust, matured and copulated, and produced viable eggs which hatched normally. The addition of 8 mM, or more, of L-cysteine proved fatal to the newly moulted worms, although premoult development and the moulting process itself were accelerated. Further modifications to the culture medium might result in faster growth rates more similar to those of *P. decipiens* and *A. simplex*.

However, it is perhaps significant that the normal development, even of eggs to the L_2 stage, is longer than that of *P. decipiens* (Burt et al. 1990) and *A. simplex* (Grabda 1976) and, accordingly, it is not surprising that later stages may grow more slowly.

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Impact of Harvesting on Frond Density and Biomass of Irish Moss (*Chondrus crispus* Stackhouse) Beds in the Southern Gulf of St. Lawrence

T. Chopin

Marine and Estuarine Research Group, Division of Sciences, University of New Brunswick, P.O. Box 5050, Saint John, N.B. E2L 4L5, Canada

and J. D. Pringle and R. E. Semple

Department of Fisheries and Oceans, Scotia–Fundy Region, Biological Sciences Branch, Halifax Fisheries Research Laboratory, P.O. Box 550, Halifax, N.S. B3J 2S7, Canada

Chopin, T., J. D. Pringle, and R. E. Semple. 1992. Impact of harvesting on frond density and biomass of Irish moss (*Chondrus crispus* Stackhouse) beds in the southern Gulf of St. Lawrence. Can. J. Fish. Aquat. Sci. 49: 349–357.

Dragrake harvesting had an adverse impact on frond density and biomass of *Chondrus crispus* Stackhouse as revealed by the comparison of these parameters in a dragraked bed (Miminegash) and a nondragraked bed (Rustico). Mean frond density peaked in September at both locations; it was significantly greater at Miminegash than at Rustico because of a higher density of small plants (Classes 1, 2, and 3). Intraannual variations in density were due to fluctuations of mostly the small plants (Classes 1 and 2). Mean frond biomass was significantly greater at Rustico than at Miminegash because of higher density and biomass of large Class 4 and 5 plants. Intraannual distribution of biomass was bimodal at Miminegash (June and September peaks) whereas it was unimodal at Rustico (peak in July). Mean frond density and biomass of reproductive plants were significantly greater at Rustico than at Miminegash. The study recommends a 1-mo delay in harvest season opening to enhance biomass yield. A longer postponement would not be beneficial, as populations will enter a period of natural decrease in density and biomass of *C. crispus* and of increased biomass of associated algae.

La pêche avec des dragues à rateaux a un impact défavorable sur la densité et la biomasse des frondes de *Chondrus crispus* Stackhouse comme l'a révélé une étude comparative de ces paramètres dans un champ exploité (Miminegash) et dans un autre non exploité (Rustico). La densité moyenne de frondes à Miminegash est significativement supérieure à celle de Rustico en raison d'une plus forte densité de petites plantes (Classes 1, 2 et 3); cette densité atteint sa valeur maximale en Septembre dans les deux sites. Les variations annuelles de densité sont dûes principalement à des fluctuations des petites plantes (Classes 1 et 2). La biomasse moyenne des frondes est significativement plus importante à Rustico qu'à Miminegash à cause des plus fortes densité et biomasse en plantes développées des Classes 4 et 5. La distribution annuelle de la biomasse est bimodale à Miminegash (pics en Juin et Septembre) alors qu'elle est unimodale à Rustico (pic en Juillet). La densité et la biomasse moyennes des plantes portant des organes de reproduction sont significativement plus élevées à Rustico qu'à Miminegash. Cette étude recommande un délai de 1 mo du début de la saison de pêche afin d'augmenter le rendement en biomasse. Un délai plus long n'offrirait aucun autre avantage, les populations entrant alors dans une période de diminution naturelle en densité et biomasse de *C. crispus* et d'augmentation de la biomasse en algues associées.

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Chondrus crispus Stackhouse (Irish moss), a common macroalga along the coast of the North Atlantic Ocean, including the Maritime provinces of Canada (McLachlan et al. 1987), exists in extensive beds in the southern Gulf of St. Lawrence and in the northern Gulf of Maine. Much of the Prince Edward Island (P.E.I.) coast consists of gently sloping sandstone ledges with *C. crispus* the numerically dominant macrophyte (Pringle and Semple 1983). Certain beds off P.E.I. have been extensively harvested annually, with heavy iron rakes towed by inshore fishing vessels, and form the basis of an important seasonal fishery (Pringle and Mathieson 1987). The fishery began in the mid-1940's and ultimately provided up to 65% of the raw material for the world's carrageenan industry as recently as 1974 (Chopin 1990). Despite new sources of raw material elsewhere, i.e. mostly the intensive Philippine farms of *Eucheuma* sp. and *Kappaphycus* sp. (Doty 1987), the present fishery still provides up to 12% of the world-wide resource.

Considerable knowledge of the biology, ecology, physiology, and biochemistry of *C. crispus* has accumulated over the years (for review, see Chopin 1986), in part because of its commercial importance. Resource science in support of resource management was begun in the late 1960's (Taylor 1973) and intensified in the 1970's (Pringle 1976) because of problems in maintaining a stable annual yield (Pringle 1986). Evidence for growth and recruitment overharvesting was presented (Pringle and Semple 1984). Recently, several studies have focused on demography (Bhattacharya 1985), frond size structure (Pringle and Semple 1988), reproductive capacity (Chopin et al. 1988), population reproductive structure (Lazo et al. 1989; Lazo and

McLachlan 1989), and plant structure (McLachlan et al. 1989). Little information, however, is available on seasonal frond density and biomass in commercially important beds within the southern Gulf of St. Lawrence. Paucity of data for these two key state variables impeded plans to upgrade resource management for western P.E.I. (Pringle and Sharp 1990).

The present study collected these needed data from dragraked and nondragraked beds of the Island's west and north coasts. The study examines the hypothesis of intraannual differences in frond density and biomass both within and between sites and investigates whether harvest yield would be enhanced by a delay in season opening date for dragraking. The study also questions the hypothesis that intense dragraking promotes high juvenile and low mature frond densities, with a concomitant reduction in frond biomass.

Materials and Methods

All Irish moss beds in Marine Plant Harvesting District (MPHD) 1 (Fig. 1) have been harvested annually since 1958. An attempt to carry out experimental harvesting on a controlled bed (Pringle 1985) was foiled by a few noncooperative fishermen. To assess harvesting impact, we were thus forced to conduct comparative studies between beds in MPHD 1 and in similar nondragraked sites beyond this district. The closest nondragraked district with similar ecological conditions (J. D. Pringle, unpubl.) is MPHD 3 (Fig. 1). The beds studied here were off Rustico, at Orby Head, where dragraking took place during 1969 and 1970 only (Craigie and Pringle 1978). Cape Gage and Pleasant View beds off Miminegash were studied in MPHD 1. These beds extend up to 1 km from shore and cover about 100 ha each (Pringle and Semple 1983).

The point transect method was chosen, with three transects at each site running perpendicular to the shore through the *C. crispus* beds. Three stations (inner, middle, and outer) were designated on each transect. Three sample units were haphazardly chosen at each station (thus, $n = 27$) by dropping a quadrat frame of 0.25 m^2 (that size deemed most efficient by Pringle (1984)) from the diver tender. The method of frond removal by hand underestimates Class 1 frond density and biomass (frond class characteristics are given below). However, the impact will be similar at both sites, considering identical collection methods and similar substrates. Samples were collected monthly during spring and summer and bimonthly thereafter. Dynamic ice conditions did not permit a January Miminegash collection. Additional sample procedures were as described previously (Chopin et al. 1988).

The plants in a sample (combination of all sample units per site per collection) were initially separated into three categories: (1) entire fronds of *C. crispus*, (2) torn fronds of *C. crispus*, and (3) "other" algae. Torn fronds, representing only 9.7 and 11.7% of the total biomass of *C. crispus* at Miminegash and Rustico, respectively, were not included in this study, as their origin was unknown. *Chondrus* fronds were then sorted into five classes, based on the number of dichotomies and frond length (Chopin et al. 1988): Class 1 = nondichotomous fronds, Class 2 = fronds with up to three dichotomies and a frond length less than 10 cm or fronds with any number of dichotomies and a frond length less than or equal to 6 cm, Class 3 = fronds with four or five dichotomies and a frond length between 6 and 10 cm, Class 4 = fronds with more than five dichoto-

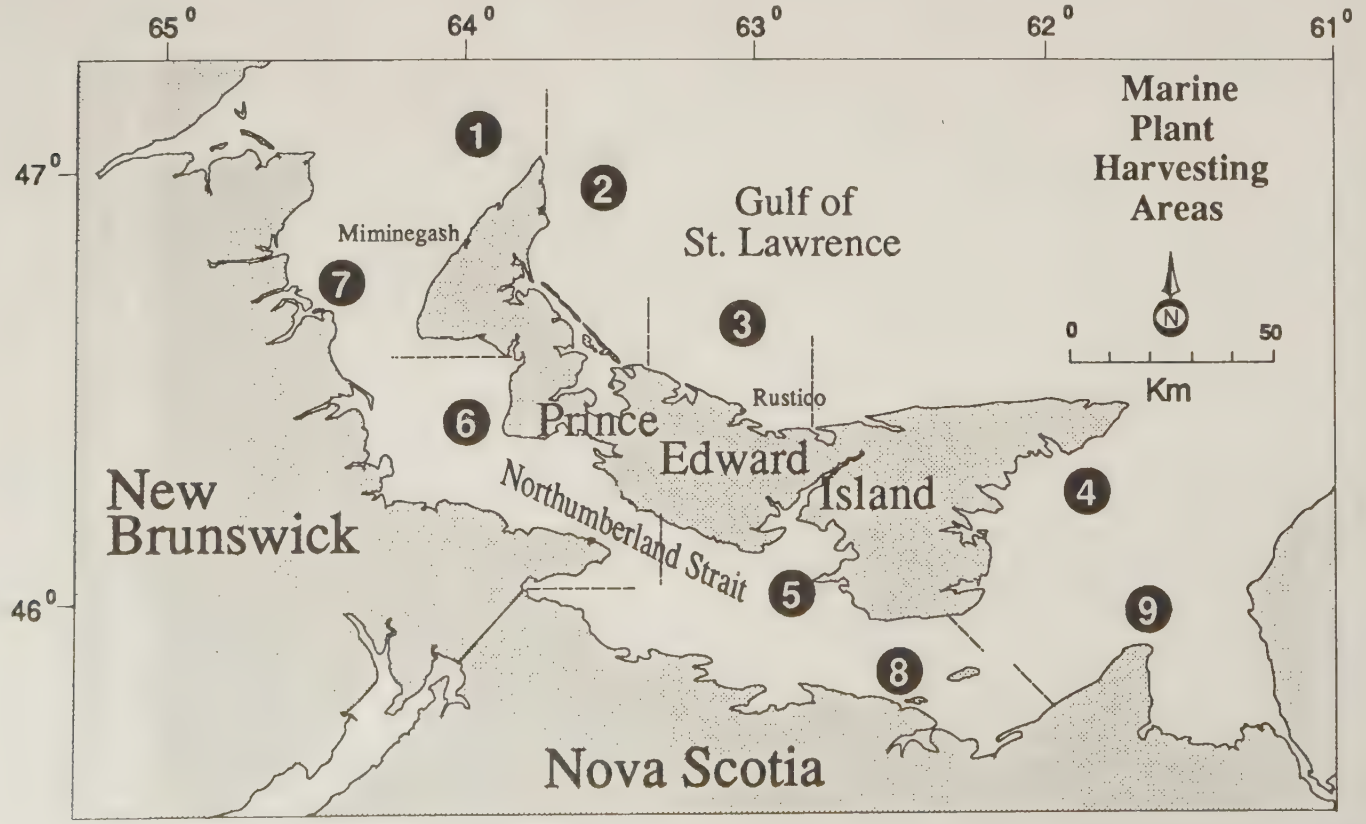

FIG. 1. Location of the studied *C. crispus* beds in Prince Edward Island, Canada: Miminegash (Marine Plant Harvesting District (MPHD) 1, dragraked) and Rustico (MPHD 3, nondragraked).

TABLE 1. Monthly and mean annual density of fronds per 0.25 m^2 and percentage of reproductive fronds of *C. crispus* at Miminegash and Rustico.

	Miminegash		Rustico	
Month	Density	% reproductive fronds	Density	% reproductive fronds
May 1985	547.6	6.2	578.8	16.0
June	603.2	11.0	514.5	11.0
July	717.6	5.0	676.5	7.8
August	983.8	2.7	701.0	4.8
September	1040.0	6.5	762.5	7.8
November	638.7	5.2	372.8	16.6
January 1986	—	—	329.2	13.6
April	365.2	6.2	361.5	9.3
Annual mean	699.5	5.8	544.7	9.8

mies and a frond length between 6 and 10 cm, and Class 5 = fronds with any number of dichotomies and a frond length greater than 10 cm. Fronds within each class were sorted into three groups: (1) reproductive female gametophytes, (2) reproductive tetrasporophytes, and (3) vegetative fronds (which, on occasion, included difficult-to-discern mature, male gametophytes). The number of fronds in each group was counted. Fronds were then dried in an oven for 72 h at 80°C and weighed to determine the biomass of each group.

Means, standard deviations, normality, and regression analyses were calculated with packaged computer programs (Nie et al. 1975). All distributions were normal; thus, the parametric Student *t*-test was employed to determine significant differences ($p \leq 0.05$) between means.

Results

Frond Density

Mean frond density for the total study period was significantly greater at Miminegash (699.5 fronds/0.25 m^2) than at Rustico (544.7 fronds/0.25 m^2). Total frond density varied from month to month within each location (Table 1), but the annual pattern for both locations was similar (Fig. 2). Frond density increased from May through September (September peaks were 1040.0 and 762.5 fronds/0.25 m^2 at Miminegash and Rustico, respectively), decreased markedly between September and November, and thereafter stabilized through April.

Frond density varied significantly amongst classes at each study site (Table 2), with the morphologically immature fronds (Classes 1 and 2) tending to have greater densities than the more mature classes (3–5), particularly at Miminegash. Class 2 fronds were the most dense at both sites; Class 5 fronds were the least dense. Large between-class decreases in frond density occurred at both sites: the drop between Classes 2 and 3 was about 73% and that between Classes 4 and 5 was about 65%. There was little within-site intraannual variation in density of the more mature fronds (Classes 3–5); the bulk of the variation was due to fluctuations of Class 1 and 2 fronds (Fig. 2). The first marked increase in Class 1 frond density at both locations occurred in July; peak densities occurred in August; thereafter, density decreased. The peak densities at Miminegash and Rustico were 303.8 and 133.3 fronds/0.25 m^2, respectively. The annual distribution of Class 2 frond densities at both locations yielded similar patterns; density increase began in July, peaking in August at Miminegash, while at Rustico the increase was 1 mo later. Peak densities were 525.3 and 465.6 fronds/0.25 m^2, respectively. The mean annual frond densities for Classes 3 and 4 at both study sites were significantly different. Class 3 frond low and high densities occurred during the early to midsummer and early to late fall, respectively, the opposite of Class 4 frond densities. There was little intraannual variation in Class 5 frond densities; however, peak densities were observed in June at Miminegash and in July at Rustico. By August, Class 5 densities had decreased by a factor of 3 at Rustico. Densities of Classes 4 and 5 also decreased in July–August at Miminegash; however, they increased in September, falling again to their minimal values during fall and winter.

The mean annual reproductive frond densities at Miminegash and Rustico were significantly different, 40.9 and 53.1 fronds/0.25 m^2, respectively, which represented 5.8 and 9.7% of the total fronds sampled (Table 3). The incidence of reproductive fronds at both study sites increased from Class 2 (no reproductive Class 1 fronds were observed) to Class 4. The density of reproductive Class 5 fronds was one half that of Class 4; however, expressed as a percentage, it was greater, particularly at Rustico. Density of reproductive fronds varied intraannually at both sites (Fig. 3). Density at Miminegash peaked in June (66.1 fronds/0.25 m^2) and September (67.3 fronds/0.25 m^2) and was minimal in August (27.1 fronds/0.25 m^2) and April (22.6 fronds/0.25 m^2); at Rustico, the peaks occurred in May (92.9 fronds/0.25 m^2) and November (61.8 fronds/0.25 m^2) and the troughs in August (33.6 fronds/0.25 m^2) and April (33.5 fronds/0.25 m^2).

Frond Biomass

The contribution of Class 1 to the total biomass at both locations was low throughout the year (Fig. 4). The maximal biomass of this class occurred in September and was higher at Miminegash (1.3 g/0.25 m^2) than at Rustico (0.5 g/0.25 m^2). The mean annual biomass of Class 1 was significantly greater at Miminegash (0.8 g/0.25 m^2) than at Rustico (0.32 g/0.25 m^2). Class 2 monthly biomass peaked in September; the biomass contributed by this class was not significantly different between the two locations, neither at the peak (14.6 versus 12.6 g/0.25 m^2) nor over the year (9.4 versus 8.1 g/0.25 m^2). Class 2 contributed a significant proportion (30–35%) of the biomass from late summer through winter at Miminegash. At this location, Classes 3, 4, and 5 had bimodal biomass distributions with peaks in June and September; the June peak was the more pronounced peak for Classes 4 and 5. Biomass distribution at Rustico was not as obvious as at Miminegash, and the peaks were at different months: two peaks (May and January) for Class 3, one peak (July) for Class 4, and two peaks (July and November) for Class 5. The annual frond biomass was significantly greater at Rustico than at Miminegash for both Class 4 (19.6 versus 13.6 g/0.25 m^2) and Class 5 (15.4 versus 10.2 g/0.25 m^2); however, the reverse was the case for Class 3 (5.2 versus 7.4 g/0.25 m^2). There was a clear bimodal distribution of total biomass, with peaks in June and September, at Miminegash; at Rustico, total biomass was high in spring and summer, with the peak in July; it decreased during fall and winter. The mean annual biomass was significantly greater at Rustico (48.6 g/0.25 m^2) than at Miminegash (41.4 g/0.25 m^2). Combined Classes 4 and 5 contributed between 34.1% (November) and 75.6% (June) of the biomass, with an annual average of 53.7%, at Miminegash (Fig. 5); the respective percentages at Rustico

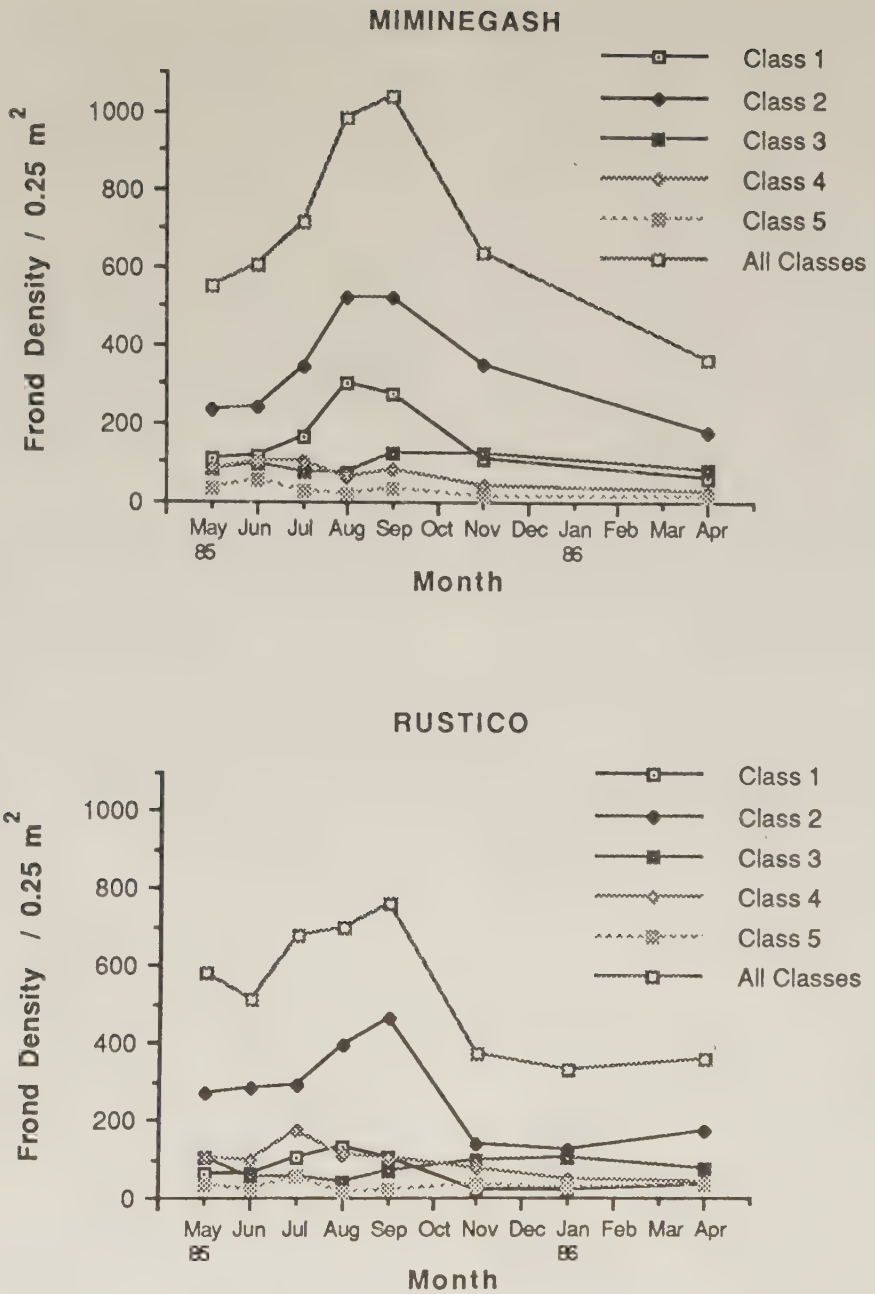

FIG. 2. Density of fronds of *C. crispus* from May 1985 to April 1986 at Miminegash and Rustico.

TABLE 2. Mean annual density of fronds of *C. crispus* per 0.25 m^2, according to the different frond classes, at Miminegash and Rustico. Asterisks indicate significant ($p \leq 0.05$) differences between the two locations.

Class	Miminegash	Rustico
Class 1	163.9*	69.8*
Class 2	341.8*	274.3*
Class 3	94.2*	73.4*
Class 4	72.1*	97.1*
Class 5	27.4	30.1
All classes	699.5*	544.7*

TABLE 3. Mean annual density of reproductive fronds per 0.25 m^2 and percentage of reproductive fronds of *C. crispus*, according to the different frond classes, at Miminegash and Rustico.

	Miminegash		Rustico	
Class	Density	%	Density	%
Class 1	0	0	0	0
Class 2	7.2	2.1	8.6	3.1
Class 3	8.4	8.9	6.4	8.7
Class 4	17.2	23.8	26.1	26.9
Class 5	8.0	29.2	12.0	39.9
All classes	40.9	5.8	53.1	9.7

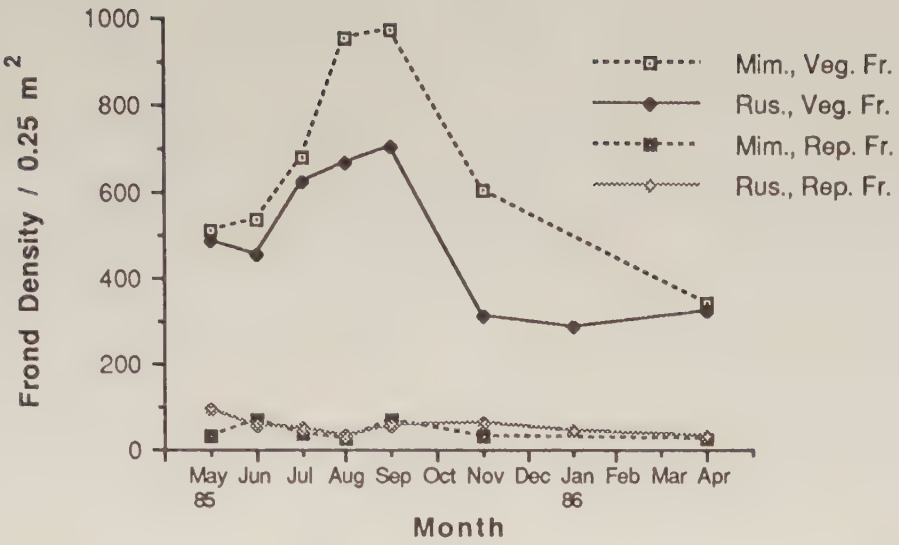

FIG. 3. Density of vegetative and reproductive fronds of *C. crispus* from May 1985 to April 1986. Mim., Miminegash; Rust., Rustico; Veg., vegetative; Rep., reproductive; Fr., frond.

were 63.6% (April), 81.7% (July), and 71% (annual average). The summer percentage drop occurred after June at Miminegash whereas it was after July and not so pronounced at Rustico.

Throughout the year, there were more vegetative than reproductive fronds (carposporangial and tetrasporangial) at both locations (Fig. 6). The mean annual biomass of both vegetative and reproductive fronds at Miminegash was 32.0 and 9.3 g/0.25 m^2, respectively; these values at Rustico were 32.7 and 15.9 g/0.25 m^2, respectively. Although the mean annual vegetative biomass was similar between the two locations, the seasonal pattern differed, being bimodal at Miminegash (peaks in June and September) and unimodal at Rustico (peak in July). The mean annual biomass of reproductive plants was significantly greater at Rustico than at Miminegash; only in June was the biomass of reproductive fronds significantly higher at Miminegash than at Rustico (22.5 versus 16.9 g/0.25 m^2). The reproductive frond biomass peaked in June and September at Miminegash versus May and September at Rustico. Reproductive frond biomass declined at both locations during summer and fall–winter; however, the decline was much more abrupt at Miminegash than at Rustico. Both mean annual biomass and percentage of reproductive fronds increased marginally from Class 1 to Class 3 and from Class 4 to Class 5; there was, however, a large increase between Class 3 and Class 4 (Table 4).

Annual variations in frond biomass followed a similar pattern to that of frond density (r varied between 0.82 and 0.94 according to the different classes and the two locations). When all classes were combined, correlations were not as strong (r = 0.60 in Miminegash; r = 0.68 in Rustico), as a large number of small plants were contributing less biomass than fewer larger plants.

The macroalgal biomass at Miminegash was largely composed of *C. crispus*, with two peaks in June and September (Fig. 7). The "other" algae (mostly *Furcellaria lumbricalis*, *Phyllophora* sp., *Corallina officinalis, Ahnfeltia plicata, Ceramium* sp., *Chordaria flagelliformis, Fucus* sp., *Halopteris scoparia, Palmaria palmata*, and *Ulva* sp.) reached a significant combined biomass in August (21.8% of the total biomass). The biomass at Rustico was also contributed to, for the most part, by *C. crispus*, with one peak in July. The biomass of the associated algae was, however, higher than at Miminegash, contributing about 25% to the August and January totals. The *C. crispus* biomass at Miminegash ranged between 84.8 and 93.8% except in summer, when it dropped to around 79%; the mean annual contribution by *C. crispus* to the total biomass was 84.9%. The mean annual contribution at Rustico was 80.2% and fluctuated between 74.7 and 86.6%.

Discussion

Seasonal variations in frond density and biomass resulted from a number of different natural phenomena as well as from harvesting where that occurred. Density of reproductive fronds was higher at Rustico than at Miminegash because of a higher density of Class 4 and 5 fronds, which had the greatest reproductive capacity (Chopin et al. 1988). At Rustico, Class 4 and 5 frond densities peaked in July (instead of June as in Miminegash) and decreased during mid- to late summer, due to natural mortality. High temperatures and low levels of nutrients during summer could account for this decrease as plants turned green and fragmented easily. Larger fronds are also at high risk due to longer exposure to attrition, epiphytism, endophytism, and herbivore grazing; these would all contribute to frond removal from the holdfast by physical forces, i.e. turbulence (Bhattacharya 1985; McLachlan et al. 1989). The mean annual frond density of *C. crispus* was greater at Miminegash than at Rustico because of a higher density of small fronds (Classes 1–3). Dragrakes have been shown to select the large frond classes (Pringle et al. 1981). Harvest season opens June 11 in Miminegash, which might explain both the marked density decrease of large fronds between June and July here and the difference in the July values between Miminegash and Rustico, where dragraking is banned. Dragrake harvesting effort decreases in July–August due to the start of the lobster fishery (Pringle and Semple 1984). Beds in Miminegash could "recover" with small fronds growing into larger ones. This might explain the increase in both Classes 4 and 5 at Miminegash in September. This explanation is in part supported by the fact that, at this

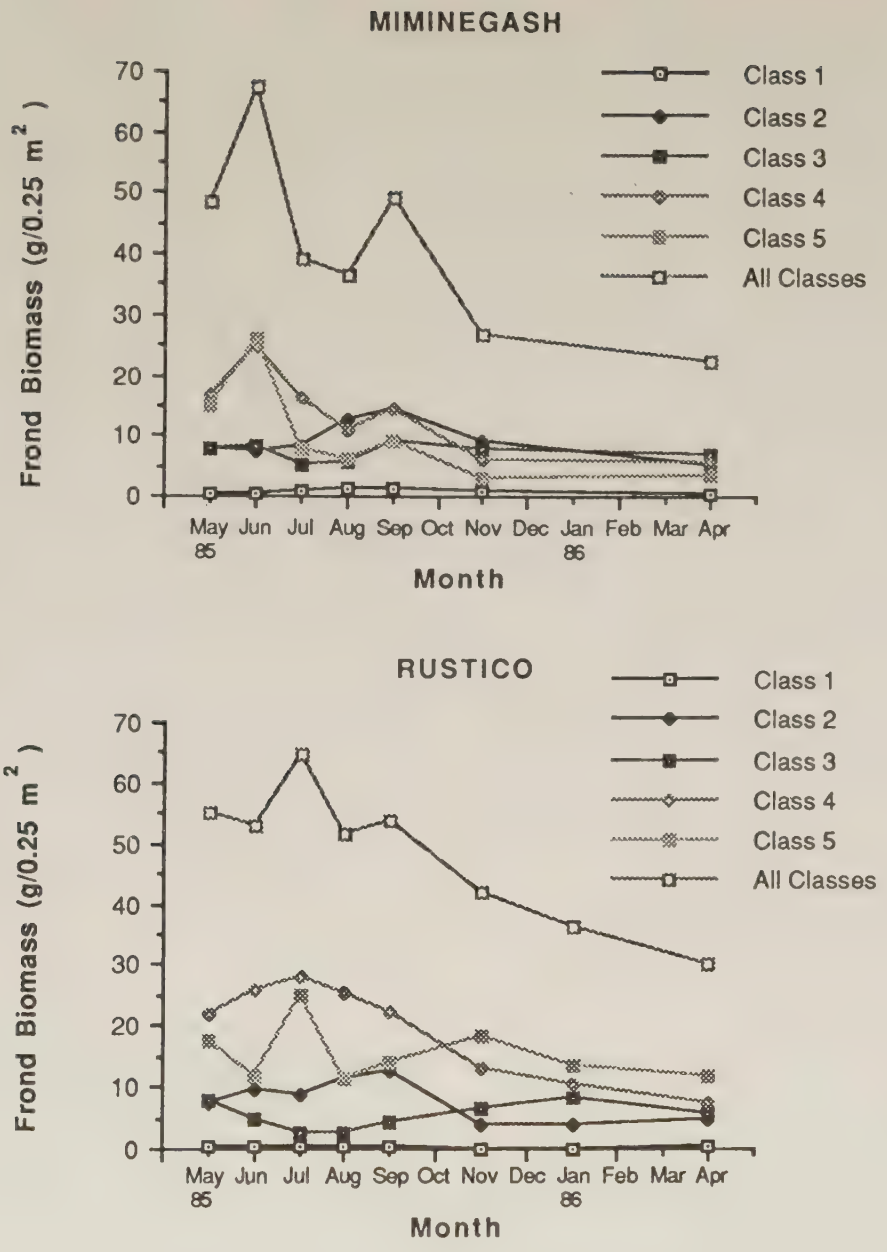

FIG. 4. Biomass of fronds of *C. crispus* from May 1985 to April 1986 at Miminegash and Rustico.

time at Rustico, the decrease of Class 4 density had slowed and that of Class 5 had increased slightly.

The intraannual variations in density which we recorded agreed with observations of others. The more pronounced variations in small fronds (Classes 1 and 2) than in less dense classes of large fronds (Classes 3–5) were also observed by Pybus (1977) in a population of *C. crispus* in Galway Bay, Ireland. There, the small intraannual variation in Class 5 frond densities indicated a stable population of large fronds where losses due to attrition, mortality, etc., were balanced by gains due to replacement by fronds developing from smaller classes. Our results also agreed with those of Cheshire and Hallam (1989) who demonstrated that density was greatest in younger populations of *Durvillaea potatorum*. Both recruitment and growth rates increased when the canopy of older plants was removed in several species of Laminariales off Nova Scotia (Smith 1986). The canopy of the larger fronds of *C. crispus* is, however, much smaller than that developed by kelps. Moreover, Taylor (1973) found that small *C. crispus* fronds did not increase in length with removal of the larger canopy-forming fronds.

Smith (1986) showed that growth was density dependent in *Laminaria longicruris* and *L. digitata*; high densities reduced growth rates. The reduction in densities between the small frond classes (1 and 2) and the large ones (3–5) shown here for *C. crispus* may be due to density effects. The weight of individual fronds was not measured in the present study. It was thus not possible to either correlate frond weight to frond density in order to assess the occurrence of density-dependent self-thinning or determine if a boundary condition was present (Yoda et al. 1963), as has been demonstrated for other monospecific seaweed stands (Cousens and Hutchings 1983). Pringle and Semple (1988) showed, however, that mean frond dry weight in nondragraked (Rustico and North Lake, P.E.I.) or lightly dragraked (Cape Jack, Nova Scotia) beds was greater, by up to a factor of 2.9, than in intensively dragraked beds (Pleasant

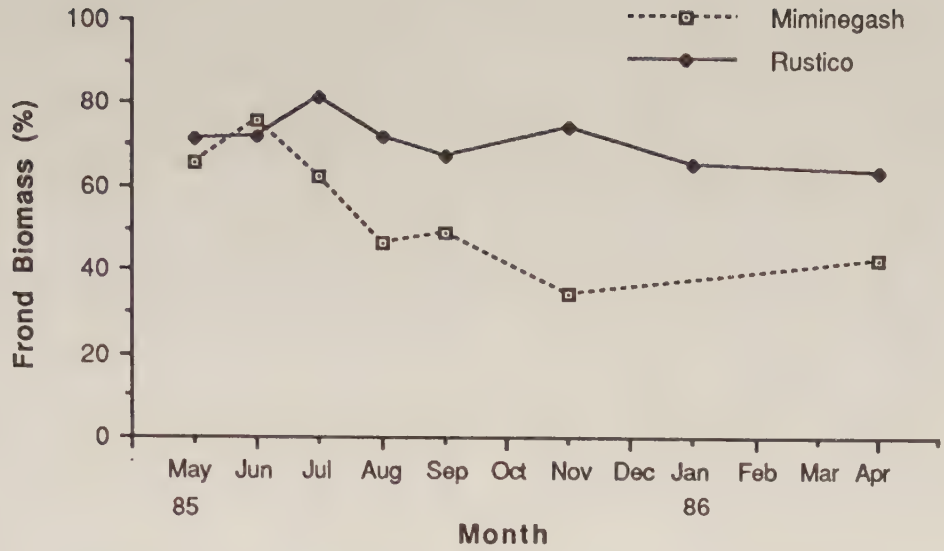

FIG. 5. Percentage of biomass of combined Class 4 and 5 fronds of *C. crispus* from May 1985 to April 1986 at Miminegash and Rustico.

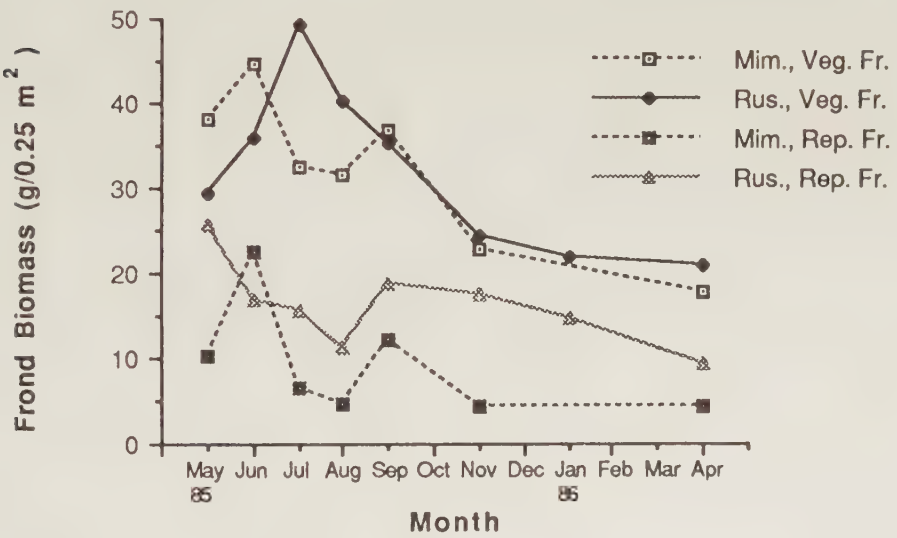

FIG. 6. Biomass of vegetative and reproductive fronds of *C. crispus* from May 1985 to April 1986 (see Fig. 3 for abbreviations).

TABLE 4. Mean annual biomass (g) of reproductive fronds per 0.25 m^2 and percentage of reproductive fronds of *C. crispus*, according to the different frond classes, at Miminegash and Rustico.

	Miminegash		Rustico	
Class	Biomass	%	Biomass	%
Class 1	0	0	0	0
Class 2	0.4	4.2	0.5	6.2
Class 3	0.9	12.9	0.6	12.0
Class 4	4.1	30.0	6.9	35.5
Class 5	3.9	38.1	7.8	50.6
All classes	9.3	22.5	15.9	32.7

View and Kenny's reefs, P.E.I.). They attributed the reduced mean frond weight to the marked increase in frequency of small fronds following harvesting.

The effect of harvesting at Miminegash on Class 4 and 5 frond biomass was similar to that on Class 4 and 5 frond density. The mean annual frond biomass was greater at Rustico than at Miminegash due to both higher density and biomass of Class 4 and 5 fronds. These classes dominated the biomass production year-round at Rustico whereas in Miminegash, they dominated only during spring and early summer. The harvest began June 11, just prior to peak biomass levels, if they were to peak in July as was observed at Rustico. Biomass decrease in August was due in part to harvesting and possibly to natural causes given the decline at Rustico. The peak of biomass in September at Miminegash coincided with one at Rustico and was likely due to a relaxation of fishing pressure at Miminegash, further evidence that Miminegash fronds would behave as those at Rustico were it not for harvesting.

The only time when the biomass of reproductive plants was higher at Miminegash than at Rustico was in June, which raises the question of what would have happened to this reproductive

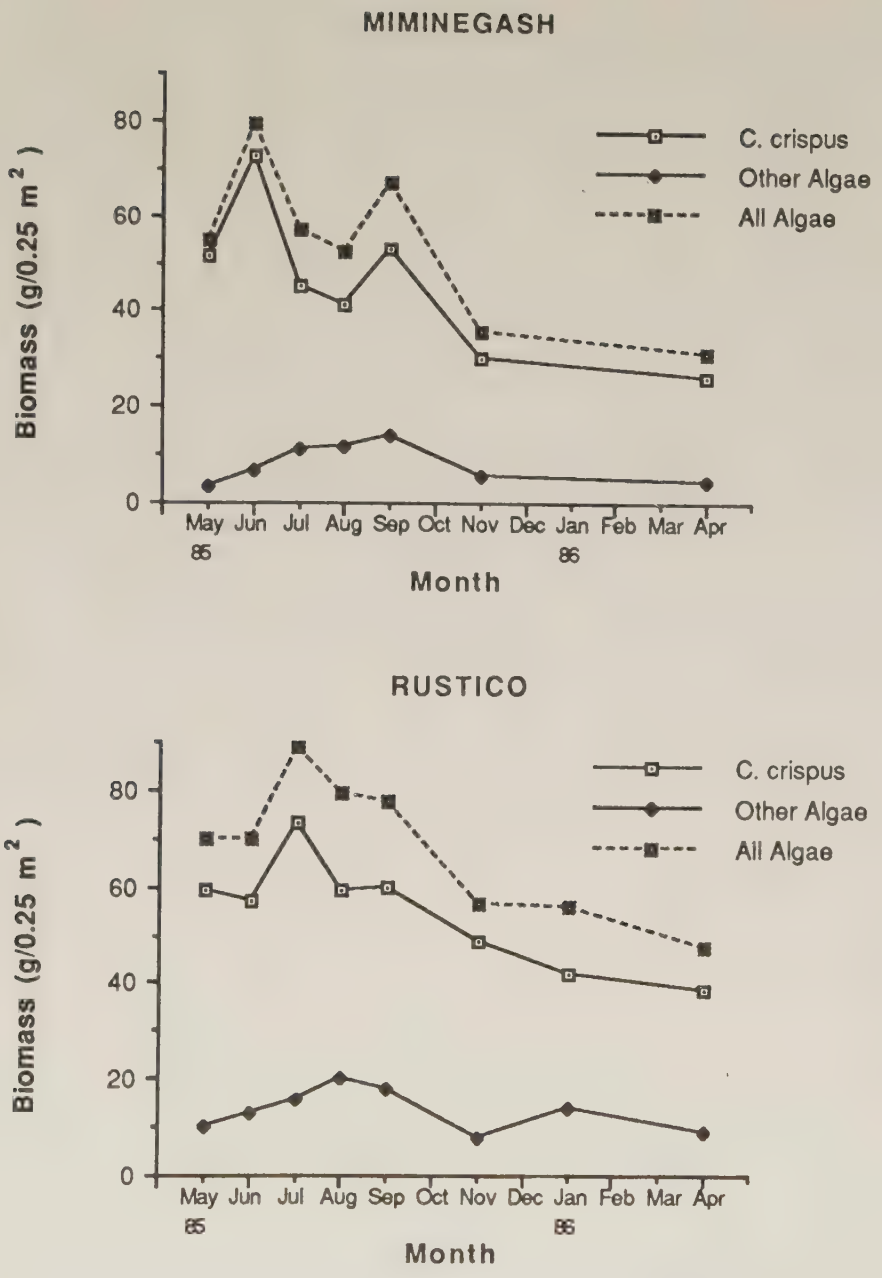

FIG. 7. Biomass of *C. crispus* (entire fronds and torn pieces) and other algae from May 1985 to April 1986 at Miminegash and Rustico.

potential, and its impact on the population structure, had dragraking not reduced it dramatically, for the rest of the year, to a level below that of Rustico. Studies on reproductive periods of *C. crispus* are numerous, sometimes conflicting, and reflect mostly geographical variations (Chopin 1986). In our case, the distribution of reproductive fronds was bimodal with peaks in late spring (May at Rustico; June at Miminegash) and late summer (September at both locations). The fact that the three generations of the life cycle of *C. crispus* could be observed throughout the year suggested that the sexual cycle is not controlled only by environmental parameters (Chen and McLachlan 1972).

The data obtained in this study corroborated the earlier observation (Pringle and Semple 1988) that dragrake harvesting at Miminegash selectively removes larger fronds (Classes 4 and 5) but allows a high productivity of the smaller fronds (Classes 1, 2, and to a lesser extent 3) to be maintained. A reverse situation existed at Rustico where the population of *C. crispus* had developed without any recent fishery impact, hence, allowing it to reach a stage of maturity, common to many plant populations, where the proportion of large individuals increase while overall population productivity decreased (Silvertown 1982). Harvesting, if its pressure is appropriately managed, can therefore be beneficial to the productivity of the beds. Moreover, our results demonstrated that the biomass of associated algae was lower throughout the year at Miminegash than at Rustico. Dragraking thus appears to enhance the purity of the Irish moss beds, which is of particular interest for the carrageenan industry, which prefers clean raw material to reduce comtamination.

Dragraking at the edge of beds might also be a method of enhancing bed dimension by eliminating those perennials

which, unlike *C. crispus*, cannot withstand extensive dragraking. This is supported by the contention of harvesters that commercial beds expand in area over the years (J. D. Pringle, unpubl.).

The results of this study support the concept that intense dragraking enhances mean annual density and reduces mean annual biomass of *C. crispus* fronds off coastal P.E.I.; it lowers these two variables when only reproductive fronds are considered. The ideal experiment to test the null hypothesis that dragraking has no significant impact on either characteristic cannot be carried out in a commercial dragraking district because of ubiquitous harvesting practices. Although differences in minor environmental state variables no doubt existed between MPHD's 1 and 3, they would have insignificant impact on the ecology of Irish moss compared with the impact of the annual dragrake harvest. Based on these data, it is thus concluded that the present harvest season opening of June 11 in MPHD 1 (Miminegash) occurs during a period of biomass increase that likely peaks in July as in the nondragraked MPHD 3 (Rustico). Consequently, these density and biomass phenological data support a study on *C. crispus* reproductive ecology (Chopin et al. 1988) in which it was recommended that the season opening date of the dragrake fishery be delayed 1 mo. A longer postponement would not be beneficial, as populations enter a period of natural decrease in both density and biomass of *C. crispus*, with a concomitant increase in the biomass of associated algae.

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Influence of Pleistocene Glaciations and Human Intervention upon Mitochondrial DNA Diversity in White Sturgeon (*Acipenser transmontanus*) Populations

James R. Brown,[1] Andrew T. Beckenbach, and Michael J. Smith

Institute of Molecular Biology and Biochemistry, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C. V5A 1S6, Canada

Brown, J. R., A. T. Beckenbach, and M. J. Smith. 1992. Influence of Pleistocene glaciations and human intervention upon mitochondrial DNA diversity in white sturgeon (*Acipenser transmontanus*) populations. Can. J. Fish. Aquat. Sci. 49: 358–367.

The influence of recent genetic bottlenecks on two Northwest Pacific populations of white sturgeon (*Acipenser transmontanus*) was studied using restriction enzyme site differences in mitochondrial DNA (mtDNA). A total of 10 mtDNA genotypes were detected in 178 sturgeon from the Columbia and Fraser rivers. Nearly 61% of individuals from both rivers had a common genotype. However, one genotype was found only in the Fraser River. Geographical substructuring was not evident in the phylogenetic analysis. The Columbia River, a Pleistocene refugium, is suggested as the source of founders for the Fraser River following the last glacial recession, about 10 000–12 000 yr ago. MtDNA genotypic frequencies were significantly different between the rivers. Estimates of mtDNA (h) and nucleotide (π) diversity were consistently higher for the recently colonized Fraser River compared with the Columbia River refugium. This condition is the reverse of all known studies of mtDNA diversity between refugium and colonized animal populations. Recent overexploitation and habitat destruction are suggested as a severe genetic bottleneck that has reduced mtDNA diversity in Columbia River sturgeon.

Les auteurs ont examiné l'influence de récents points d'étranglement génétiques sur deux populations d'esturgeon blanc (*Acipenser transmontanus*) du Pacifique nord-ouest en établissant les différences du site d'hydrolyse de l'ADN mitochondrial (ADNmt) par des endonucléases de restriction. Ils ont ainsi relevé 10 génotypes de l'ADNmt chez 178 esturgeons des fleuves Columbia et Fraser. Presque 61 % des individus de ces deux cours d'eau partageaient le même génotype. Par contre, un seul génotype a été identifié dans le fleuve Fraser. Une sous-structure géographique n'a pas été révélée par une analyse phylogénétique. Selon les auteurs, le fleuve Columbia, un refuge du Pléistocène, serait la source des colonisateurs du fleuve Fraser qui s'y auraient dispersés après la dernière récession glaciaire, il y a de cela environ 10 000 à 12 000 années. Les fréquences génotypiques de l'ADNmt étaient nettement différentes entre les rivières. Les estimations de la diversité de l'ADNmt (h) et des nucléotides (π) étaient régulièrement plus élevées dans le cas du fleuve Fraser récemment colonisé par rapport au refuge du Columbia. Cette situation va à l'encontre de toutes les études de la diversité de l'ADNmt entre des populations d'un refuge et d'un site colonisé. Selon les auteurs, la récente surexploitation et la destruction de l'habitat sont à la source d'un grave point d'étranglement génétique qui a réduit la diversité de l'ADNmt chez l'esturgeon blanc du fleuve Columbia.

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Mitochondrial DNA (mtDNA) has been widely used as a genetic marker in population studies because of its higher mutation rate relative to nuclear DNA, generally maternal inheritance, and lack of sexual recombination (reviewed in Brown 1985). Several North American fish species have been subjected to mtDNA analysis including walleye (*Stizostedian vitreum*; Ward et al. 1989), American shad (*Alosa sapidissima*; Bentzen et al. 1989), lake whitefish (*Coregonus clupeaformis*; Bernatchez et al. 1989), and chinook salmon (*Oncorhynchus tshawytscha*; Wilson et al. 1987).

In the above studies, the northern distributions of intraspecific mtDNA genotypes suggested possible dispersal patterns of founder groups from one or several southern refugia. Southern river drainages, located beyond the maximum excursions of Pleistocene ice sheets, have had prolonged periods of ecological stability. In contrast, northern freshwater habitats have been completely deglaciated for only the last 10 000–12 000 yr (Smith 1981). Postglacial dispersal from southern refugia was probably a severe genetic bottleneck, which has typically lead to a reduction in mtDNA diversity among northern populations of widely distributed species. In this study, we describe mtDNA variation between two populations of white sturgeon (*Acipenser transmontanus*) from refugium and formerly glaciated river systems.

Sturgeon and their closest living relatives, paddlefish, are members of the Infraclass Chondrostei, a pre-Jurassic diversion from the teleost lineage (Lauder and Liem 1983; Gardiner 1984). Knowledge of the life history and population genetics of sturgeon species, particularly *A. transmontanus*, is sparse. The white sturgeon is the largest freshwater fish in North America, and individuals may exceed 600 kg in weight and 6 m in length (Scott and Crossman 1973). Reproductive maturity is

[1]Author to whom correspondence should be addressed. Present address: Department of Biochemistry, Sir Charles Tupper Medical Building, Dalhousie University, Halifax, N.S. B3H 4H7, Canada.

attained between ages 11 and 34 yr and specimens older than 70 yr have been recorded (Semakula and Larkin 1968).

Acipenser transmontanus is anadromous and readily moves between freshwater and marine coastal habitats. Conventional and radiotagging studies have yielded little information regarding either the length of time sturgeon spend at sea, their fidelity to river of birth, or migration patterns (Miller 1972a, 1972b; Haynes and Gray 1981; Haynes et al. 1978; Dixon 1986), although individuals may travel distances of several hundred kilometres in marine coastal waters (Galbreath 1985). Because sturgeon are capable of repeated spawnings, the potential exists for significant gene flow among sturgeon populations with access to the Pacific Ocean.

The Fraser and Columbia rivers have the largest *A. transmontanus* populations in the Pacific Northwest (McPhail and Lindsay 1986). Both rivers empty into the Pacific Ocean, with the mouth of the Fraser River located about 500 km north of that of the Columbia River. The southern two thirds of the Columbia River remained ice-free during the Pleistocene glaciations (McPhail and Lindsay 1986). In comparison, the entire Fraser River was completely covered by the Cordilleran Ice Sheet as recently as 10 000–12 000 yr ago (Clague and Luternauer 1983). Based on the paleogeography of the region, it is probable that Columbia River sturgeon migrating northward through marine coastal waters colonized the Fraser River after the last glacial retreat. Therefore, this founder effect may have acted as a significant genetic bottleneck and caused notably lower mtDNA diversity in the Fraser River sturgeon population relative to the Columbia River population.

Human activity may have imposed a second genetic bottleneck of unknown magnitude. Nearly all North American sturgeon populations were extensively overfished from the late 1800's to the early 1900's (reviewed in Binkowski and Doroshov 1985). Later construction of large hydroelectric dams on the Columbia River in the 1930's and 1950's may have accentuated this bottleneck effect through alteration of migration routes and destruction of spawning habitat (Galbreath 1985).

In this study, mtDNA variation was determined in *A. transmontanus* populations of the Fraser and Columbia rivers over a 3-yr period (i) to investigate within-river substructuring of populations, (ii) to provide estimates of existing genetic diversity in light of recent bottleneck events, and (iii) to determine the extent of past and contemporary gene flow among populations.

Materials and Methods

Sample Collection and mtDNA Isolation

mtDNA was extracted from fresh sturgeon liver tissue removed in the field and transported on ice to the laboratory. mtDNA's were prepared from 105 sturgeon caught in the Fraser River, British Columbia, and 73 sturgeon from the Columbia River, Washington State. Most sturgeon were between 1 and 2 m fork length, with estimated ages ranging from 9 to 30 yr (D. Lane, Malaspina College, Nanaimo, B.C., unpubl. data).

State of Washington Department of Fisheries personnel collected liver samples from commercially caught sturgeon in the late summers of 1987 and 1988 and the spring of 1990. Sturgeon were caught near the mouth of the lower Columbia River (n = 19) and in the upper Columbia River (n = 54) above Bonneville Dam, located about 230 km from the river mouth. This dam is the last impoundment of the Columbia River before it flows into the Pacific Ocean and has effectively isolated upper river sturgeon populations since the mid-1930's. Upper Columbia River sturgeon were caught from three separate reservoirs (in order upstream from Bonneville Dam): Bonneville Pool (n = 25), The Dalles Pool (n = 24), and John Day Pool (n = 5). The latter two pools were created by dams built in the 1950's.

Commercial sturgeon fishing is prohibited on the Fraser River, so most of the liver samples came from sturgeon caught by the seasonal (May–November) sport fishery. A few sturgeon were caught either incidentally in salmon gill nets or in a separate tagging study (D. Lane, pers. comm.). Sample sites ranged from the mouth of the Fraser River to the town of Lillooet, nearly 320 km upstream. The largest sample was taken in 1987 (n = 66), with smaller samples in 1988 (n = 19) and 1989 (n = 20). It is not known whether the lower sample sizes in latter years reflect changes in the sturgeon population or a reduction in angler effort.

A single mtDNA sample was also prepared from liver tissue, pooled from 20 sibling juvenile sturgeon (2.5 mo old) from the Sacramento River, California.

mtDNA was extracted from liver tissue within 6–72 h of collection. mtDNA was isolated using a modified protocol of Lansman et al. (1981) that omitted the sucrose gradient step but included an increase in CsCl – ethidium bromide centrifugation time to 60–72 h. Typical mtDNA yields were 300–500 ng·g homogenized tissue^{-1}.

mtDNA Analysis

Comparisons were based on digests with 12 restriction enzymes with specificities for six-nucleotide (*Apa*I, *Bcl*I, *Bgl*II, *Eco*RI, *Hind*III, *Pvu*II, and *Xba*I), multiple six-nucleotide (*Acc*I, *Ava*I, *Hae*II, and *Hinc*II), and multiple five-nucleotide (*Ava*II) recognition sequences. The mtDNA genome of one individual was digested with paired combinations of several enzymes (*Ava*I, *Bcl*I, *Eco*RI, *Hind*III, *Kpn*I, *Pvu*II, *Sac*I, and *Xba*I) to map the relative locations of specific restriction sites. Enzyme digest conditions were as specified by the vendors (BRL and Pharmacia). DNA fragments were visualized under ultraviolet light after electrophoresis in ethidium bromide stained agarose gels.

The observed intraspecific differences in DNA fragment patterns for each enzyme were due to relatively few restriction site changes. Therefore, it was possible to base divergence estimates on the presence or absence of restriction sites located on specific DNA fragments. Differences in fragment length caused by the loss or gain of restriction sites were distinguished from length polymorphisms found in the control or D-loop region of *A. transmontanus* mtDNA (Buroker et al. 1990). Southern blot analysis and direct sequencing identified DNA fragments containing the control region in specific restriction enzyme digests (Brown 1991). In the phylogenetic analysis, mtDNA genotypes were defined by the loss or gain of restriction enzyme sites. Phylogenetic analysis based on mtDNA length variation is inappropriate because the mechanism and rate of mutations for the addition or deletion of repeat sequences in the control region are considerably different from those of nucleotide substitutions and may not be entirely neutral (Rand and Harrison 1989; Buroker et al. 1990; Brown 1991).

Nucleotide substitutions per site (δ) between pairs of mtDNA genotypes were estimated using the maximum likelihood

method of Nei and Tajima (1983). A UPGMA tree of percent sequence divergence (d) was constructed with internal nodes plotted as $1/2d$ (Nei 1987, p. 293–296). Standard errors of internal nodes were calculated using the method of Nei et al. (1985).

The presence or absence of restriction sites was also analyzed using the Wagner parsimony method. The bootstrapped mixed parsimony algorithm (BOOT) of the PHYLIP 3.3 program package was used with 100 replicates (Felsenstein 1990). The estimate of haplotypic or nucleon diversity (h) of Nei and Tajima (1981), used here to evaluate the diversity of mtDNA lineages within a population, was calculated as

$$h = n\left(1 - \sum_{i=1}^{r} x_i^2\right)/(n - 1)$$

where x_i is the frequency of the ith type of mtDNA in a population of n specimens and r is the number of mtDNA types. Although this index is usually applied to the heterozygosity at nuclear loci, it is an appropriate measure for the diversity of maternal lineages (Nei 1987). Its application here permits comparisons with published mtDNA diversity estimates for other species.

A second estimate of nucleotide diversity (π) was calculated as

$$\pi = \sum_{ij} x_i x_j \pi_{ij}$$

where x is the frequency of the ith and jth type of mtDNA in a population and π_{ij} is the percent sequence difference between the two types (Nei and Li 1979). Therefore, this index of nucleotide diversity considers the frequency of occurrence of mtDNA types in a population as well as the divergence among mtDNA lineages.

Sensitivity of the sampling regime to detect mtDNA genotypes was evaluated using the combinatorial analysis of Hebert et al. (1988) as applied by Bernatchez et al. (1989). The relationship between sample size and the number of mtDNA genotypes observed in the Fraser and Columbia rivers was determined from the cumulative number of mtDNA genotypes detected in a random choice of 60 individuals. Increments of three individuals (n = 3, 6, 9, 12, . . ., 60) were selected at random, without replacement, using the random case selection algorithm in the data management program (P1D) of the BMDP statistical software package (Dixon 1983). The mean number of genotypes detected in 10 randomizations was plotted against sample size.

Results

Restriction Map of Sturgeon mtDNA

The size of *A. transmontanus* mtDNA varies between 16.1 and 16.7 kb depending on the number of tandemly repeated 82 nucleotide sequences in the D-loop region (Buroker et al. 1990). Figure 1 shows several restriction enzyme sites, the D-loop region, and genes for apocytochrome *b*, $tRNA^{Thr}$, and $tRNA^{Pro}$ (Gilbert et al. 1988; Brown et al. 1989; Buroker et al. 1990). The order and transcriptional polarity of these genes are identical to that of other vertebrates.

Intraspecific Phylogeny

The 12 restriction enzymes detected between 52 and 56 sites per *A. transmontanus* mtDNA genome. Because most of the

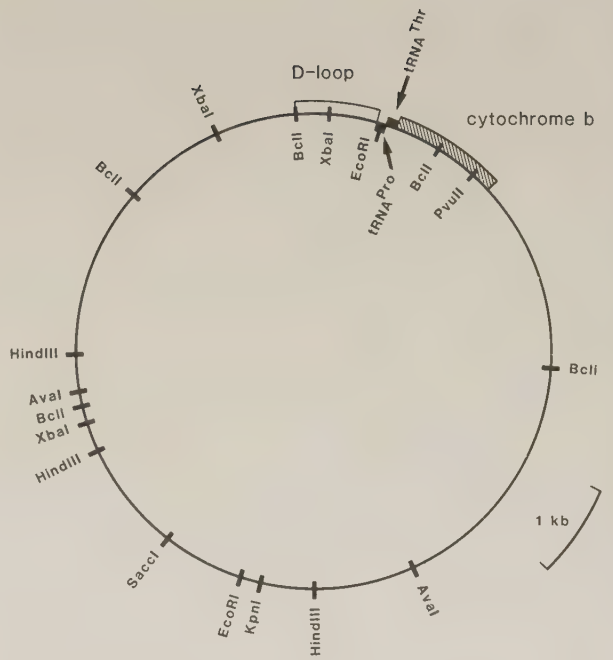

FIG. 1. Restriction enzyme map of the *A. transmontanus* mtDNA genome showing the locations of known coding sequence.

enzymes cleaved at six-nucleotide recognition sites, between 285 and 323 nucleotides or 1.8–2.0% of the genome was sampled. DNA fragment lengths for each enzyme are presented in Appendix A. Nearly 50% of individuals were heteroplasmic (i.e. had multiple copies of different mtDNA types within an individual) for length variation. This length variation was localized to the control region using a cloned 0.8-kb fragment of the entire control region from *A. transmontanus* mtDNA to probe Southern blots (Buroker et al. 1990; Brown 1991).

Five of the 12 restriction enzymes used in the phylogenetic analysis (*Acc*I, *Ava*I, *Ava*II, *Hinc*II, and *Bcl*I) revealed intraspecific restriction site polymorphisms in *A. transmontanus*. The loss or gain of single restriction sites was observed for the enzymes *Acc*I, *Ava*II, and *Hinc*II (Appendix A). There were two predominant *Ava*I fragment patterns, with a third pattern occurring in a single individual. Three individuals had a parallel loss and gain of two different *Bcl*I sites relative to the most frequent *Bcl*I fragment pattern.

In total, 11 mtDNA genotypes or clonal lines (based on the loss or gain of restriction sites) were detected in *A. transmontanus*. Of the 179 individuals examined, 109 (61%) shared a single mtDNA genotype, At2 (Fig. 2). The next three most common mtDNA genotypes (At1, At3, and At4) occurred in 13, 21, and 22 individuals, respectively. The seven remaining genotypes occurred in one to seven individuals.

Sequence divergence between genotypes ranged between 0.17 and 1.07% (Table 1). UPGMA analysis revealed three major genotypic clusters (Fig. 2). Genotypes At1, At3, At4, At5, and At6 formed one cluster. The branch ordering of four of these genotypes is arbitrary because At1, At4, and At5 are equally diverged from At6. The second major cluster consists of At2, At7, and At8. The third cluster, as defined by two *Bcl*I restriction site differences, included three rare genotypes (At9, At10, and AT11). Low estimates of intraspecific sequence divergence resulted in overlapping standard errors about the internal nodes. Therefore, the topology of the UPGMA tree is not significant.

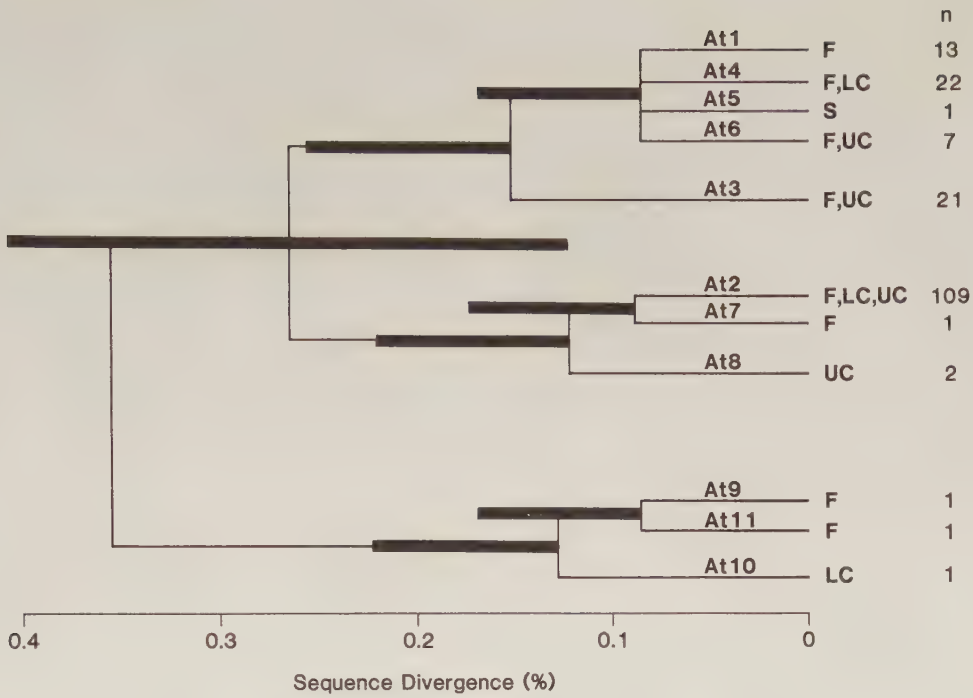

FIG. 2. UPGMA phylogeny of *A. transmontanus* genotypes (At1–11). Branching points are plottted as 1/2*d*. Bars represent 1 SE on either side of the branching point. Number of individuals (*n*) and occurrence in the Fraser (F), Lower Columbia (LC), Upper Columbia (UC), and Sacramento (S) rivers are shown.

TABLE 1. Percent pairwise sequence divergence estimates (lower diagonal) and standard errors (upper diagonal) for mtDNA lineages of *A. transmontanus* (At1–11).

	At1	At2	At3	At4	At5	At6	At7	At8	At9	At10	At11
At1	—	0.355	0.171	0.242	0.242	0.168	0.397	0.308	0.297	0.302	0.349
At2	0.708	—	0.308	0.246	0.246	0.302	0.174	0.177	0.397	0.404	0.355
At3	0.173	0.533	—	0.171	0.302	0.242	0.355	0.251	0.349	0.247	0.302
At4	0.344	0.350	0.173	—	0.242	0.168	0.302	0.308	0.297	0.302	0.242
At5	0.344	0.350	0.524	0.344	—	0.168	0.302	0.308	0.297	0.397	0.297
At6	0.170	0.523	0.344	0.170	0.170	—	0.349	0.355	0.238	0.349	0.297
At7	0.881	0.176	0.708	0.523	0.523	0.695	—	0.251	0.435	0.443	0.397
At8	0.533	0.179	0.357	0.533	0.533	0.708	0.357	—	0.443	0.362	0.404
At9	0.514	0.880	0.695	0.514	0.514	0.338	1.051	1.072	—	0.242	0.168
At10	0.523	0.897	0.351	0.523	0.886	0.695	1.072	0.721	0.344	—	0.171
At11	0.692	0.707	0.523	0.344	0.695	0.514	0.880	0.897	0.170	0.173	—

A closely related species, *A. medirostris*, was used as an outgroup taxon in the Wagner parsimony analysis. Presence or absence data on a total of 64 restriction sites were entered into the analysis (Appendix A). In 100 bootstrap replicates, only one intraspecific branching point occurred more than 50 times (Fig. 3). However, the topology of the parsimony tree was generally congruent with that of the UPGMA tree, with similar clusterings of genotypes At2, At7, and At8 and genotypes At9, At10, and At11.

Distinctive geographical groupings of Fraser and Columbia River genotypes were not evident in either phylogenetic analysis. The pooled sibling sample from the Sacramento River was a separate genotype (At5) that clustered closely with genotypes found in the Fraser and Columbia rivers. In the nomenclature used in Table 2, the composite genotype of this California sample was ABABA.

Geographical Occurrence of Genotypes

The preceding phylogenetic analysis showed that topologies of the two genotypic trees were not statistically significant. Genotypes were characterized on the basis of the presence or absence of restriction sites which indicates actual nucleotide sequence changes between the mtDNA genomes of individuals. Therefore, analysis of genotypic frequencies is justified even though the inferred ancestral relationships between genotypes are not statistically well supported.

The geographical range of the most frequent genotype (At2) extended throughout the sampled range in the Columbia and Fraser rivers (Fig. 4 and 5; Table 2). However, the observed frequency of all genotypes was significantly different between river systems (log-likelihood ratio chi-square or $G = 36.84$, $df = 5$, $p < 0.0001$). Genotypic frequencies were also distinctive between the upper and lower Columbia River ($G = 21.99$, $df = 5$, $p < 0.001$).

Genotype At2 was found throughout the Columbia River. However, less frequent genotypes were specific to areas above (At3, At6, and At8) or below (At4 and At10) Bonneville Dam (Fig. 4). Two of the rare genotypes (At8 and At10) were unique to the Columbia River.

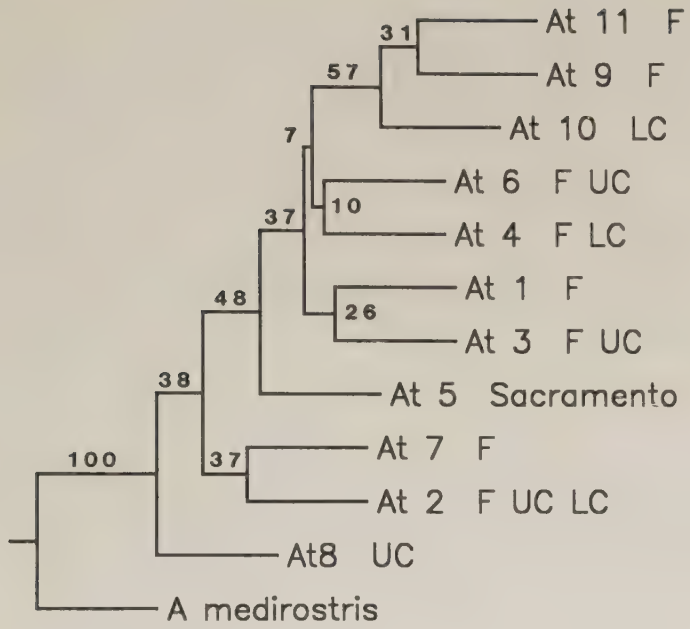

FIG. 3. Wagner parsimony phylogeny of *A. transmontanus* genotypes. The number of times each branching point occurred in 100 bootstrap replications is shown. Letter codes for geographical location of genotypes follow Fig. 2.

In the Fraser River, the most common genotypes (At2, At3, and At4) were distributed throughout the middle and lower sections of the river (Fig. 5). In the nine individuals sampled upstream of Hell's Gate Rapids (280–320 km from the river mouth), only genotypes At2 (n = 8) and At4 (n = 1) were detected. The Hell's Gate Rapids, located 200 km upstream, have completely isolated upper Fraser River sturgeon populations since 1912. In comparison, eight separate genotypes were detected downstream of the rapids.

Only one genotype (At1) had a narrow distribution. This unique genotype was found in 13 individuals caught in a 45-km-long section of the Fraser River, 90 km from the river mouth (Fig. 5). This genotype was represented in roughly the same proportions in samples collected over a 3-yr period.

mtDNA Diversity

Both estimators of mtDNA diversity showed that mtDNA diversity in the Fraser River (h = 0.70, π = 0.157) is nearly twice that in the Columbia River (h = 0.36, π = 0.076) (Table 3). In the Columbia River, greater mtDNA diversity was found below (h = 0.49, π = 0.103) than above (h = 0.30, π = 0.067) Bonneville Dam. MtDNA diversity in the upper Columbia River was considerably lower in the Bonneville Pool in comparison with The Dalles and John Day pools. Lower mtDNA diversity (h = 0.22) was also estimated for isolated sturgeon populations above Hell's Gate Rapids (Fig. 5) compared with the lower Fraser River (h = 0.72).

Diversity estimates for the Fraser River derived from annual subsamples in 1987 (n = 66), 1988 (n = 19), and 1989 (n = 20) were consistently higher than estimates for the Columbia River (n = 73), suggesting that low diversity estimates for the Columbia River population were not artifacts of sample size.

Sample Size and Detection of Genotypes

In the combinatorial analysis, the number of genotypes detected rose with sample size until an asymptote was reached for samples larger than 36–39 individuals (Fig. 6). Fewer genotypes were detected in the Columbia River (four to five genotypes) compared with the Fraser River (five to six genotypes).

Discussion

Intraspecific Phylogeny

The phylogenetic analysis indicates that *A. transmontanus* mtDNA genotypes are little diverged and generally lack any significant geographical substructure. Low confidence intervals around intraspecific branching points can be attributed to either a deficiency in the number of nucleotides sampled or historical events that have greatly reduced mtDNA diversity in this species.

An examination of the recognition sites of the polymorphic enzymes, *Acc*I (GT[$^{AG}_{CT}$]AC), *Ava*I (GPyCGPuG), *Ava*II (GG[$^{A}_{T}$]CC, and *Hinc*II (GTPyPuAC), reveals that all have multiple recognition sequences. Of the fixed hexameric enzymes used (*Apa*I, *Bcl*I, *Bgl*II, *Eco*RI, *Hind*III, *Pvu*II, and *Xba*I), only *Bcl*I was polymorphic, with three individuals displaying a variant fragment pattern. In other studies of intraspecific mtDNA variation, including some of the fish population studies cited here, fixed hexameric enzymes were polymorphic. DNA sequence comparisons of a hypervariable

TABLE 2. MtDNA genotypic frequencies of *A. transmontanus* for entire samples from the Fraser and Columbia rivers as well as for various subsamples of the Columbia River. The letter code in parentheses denotes polymorphic DNA fragment patterns for the enzymes *Bcl*I, *Hinc*II, *Ava*I, *Ava*II, and *Acc*I.

	Entire rivers		Columbia		Upper Columbia pools		
Genotype	Fraser	Columbia	Lower	Upper	Bonneville	The Dalles	John Day
n	105	73	19	54	25	24	5
At1 (AAABB)	0.12	0.0	0.0	0.0	0.0	0.0	0.0
At2 (ABBAA)	0.49	0.80	0.68	0.83	0.92	0.75	0.80
At3 (AABBB)	0.18	0.03	0.0	0.04	0.04	0.04	0.0
At4 (ABBBB)	0.16	0.07	0.26	0.0	0.0	0.0	0.0
At6 (ABABB)	0.02	0.07	0.0	0.09	0.04	0.17	0.0
At7 (ABCAA)	0.01	0.0	0.0	0.0	0.0	0.0	0.0
At8 (AABAA)	0.0	0.03	0.0	0.04	0.0	0.04	0.20
At9 (BBABB)	0.01	0.0	0.0	0.0	0.0	0.0	0.0
At10 (BABBB)	0.0	0.01	0.05	0.0	0.0	0.0	0.0
At11 (BBBBB)	0.01	0.0	0.0	0.0	0.0	0.0	0.0

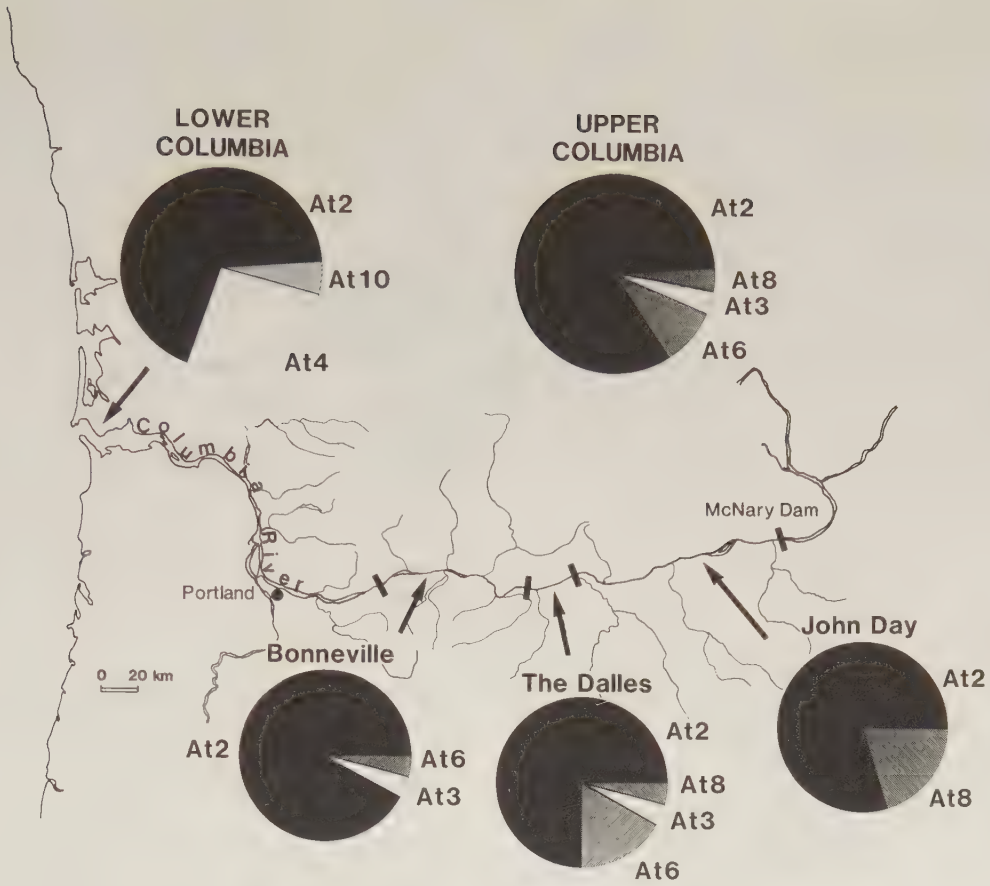

FIG. 4. MtDNA genotypic frequencies in the Columbia River. Upper pie charts show frequencies in the lower (n = 19) and upper (n = 54) Columbia River as delineated by the Bonneville Dam. Bottom pie charts subdivide genotypic frequencies of the upper Columbia sample into reservoirs: Bonneville Pool (n = 25), The Dalles Pool (n = 24), and John Day Pool (n = 5). Solid bars across the river show the location of each dam.

segment in the control region of *A. transmontanus* mtDNA generally concur with the low intraspecific divergence estimates obtained from restriction enzyme data (Brown 1991). The low levels of mtDNA sequence variation detected in *A. transmontanus* are thus probably reflective of the lack of sequence differences among individuals rather than inadequate sampling of nucleotides or populations (see below).

Low genetic variability has been noted for other North American chondrosteans. Little intraspecific variation was observed in a protein electrophoretic study of paddlefish (*Polyodon spathula*) populations (Carlson et al. 1982). Two other species of sturgeon, *Scaphirhynchus albus* and *S. platorynchus*, were indistinguishable at 37 protein loci (Phelps and Allendorf 1983). Restriction enzyme analysis has revealed low intraspecific mtDNA variation in northern populations of the sturgeon species, *A. fulvescens*, *A. medirostris*, and *A. oxyrhynchus* (Brown 1991). Populations of *A. oxyrhynchus* in the southeastern United States also show very low mtDNA diversity (Bowen and Avise 1990).

Postglacial dispersal and possibly more contemporary migrations would minimize geographical substructuring of *A. transmontanus* mtDNA genotypes, while recent, severe genetic bottleneck effects would reduce mtDNA diversity. Bidirectional gene flow between the Fraser and Columbia rivers could be occurring, although the fidelity of migratory *A. transmontanus* to natal rivers is unknown. Sturgeon tagged in the Columbia River have been recaptured in marine waters off the coast of northern Washington State (Galbreath 1985). A low number of migratory female spawners per generation could prevent significant divergence of mtDNA genotypes between these river systems (Slatkin 1987).

The most geographically segregated sturgeon mtDNA genotype was At1. In 3-yr of study, all individuals with this genotype were caught in a 45-km section of the lower Fraser River. This genotype (n = 13) comprised about 12% of the Fraser River sample. The restricted range of At1 and higher divergence (>1.0%) between certain conspecific genotypes (Table 1) suggest the existence of moderate isolation processes. Discussion regarding reproductive barriers is highly speculative given our limited understanding of sturgeon behavior. Potential microgeographic isolation of spawning females could occur in two large lakes draining into the Fraser River. Sturgeon may also be segregated into nonsynchronous spawning cohorts separated by reproductive cycles of several years (Roussow 1957; Semakula and Larkin 1968; Doroshov 1985).

mtDNA Diversity

Previous studies have consistently shown that intraspecific mtDNA diversity (h) is lower in recently founded populations relative to populations in areas of long-term ecological stability (Table 3). As an example, North American lake whitefish populations in recently (<18 000 yr ago) deglaciated rivers had much lower diversity (h = 0.26 ± 0.25) than Eurasian pop-

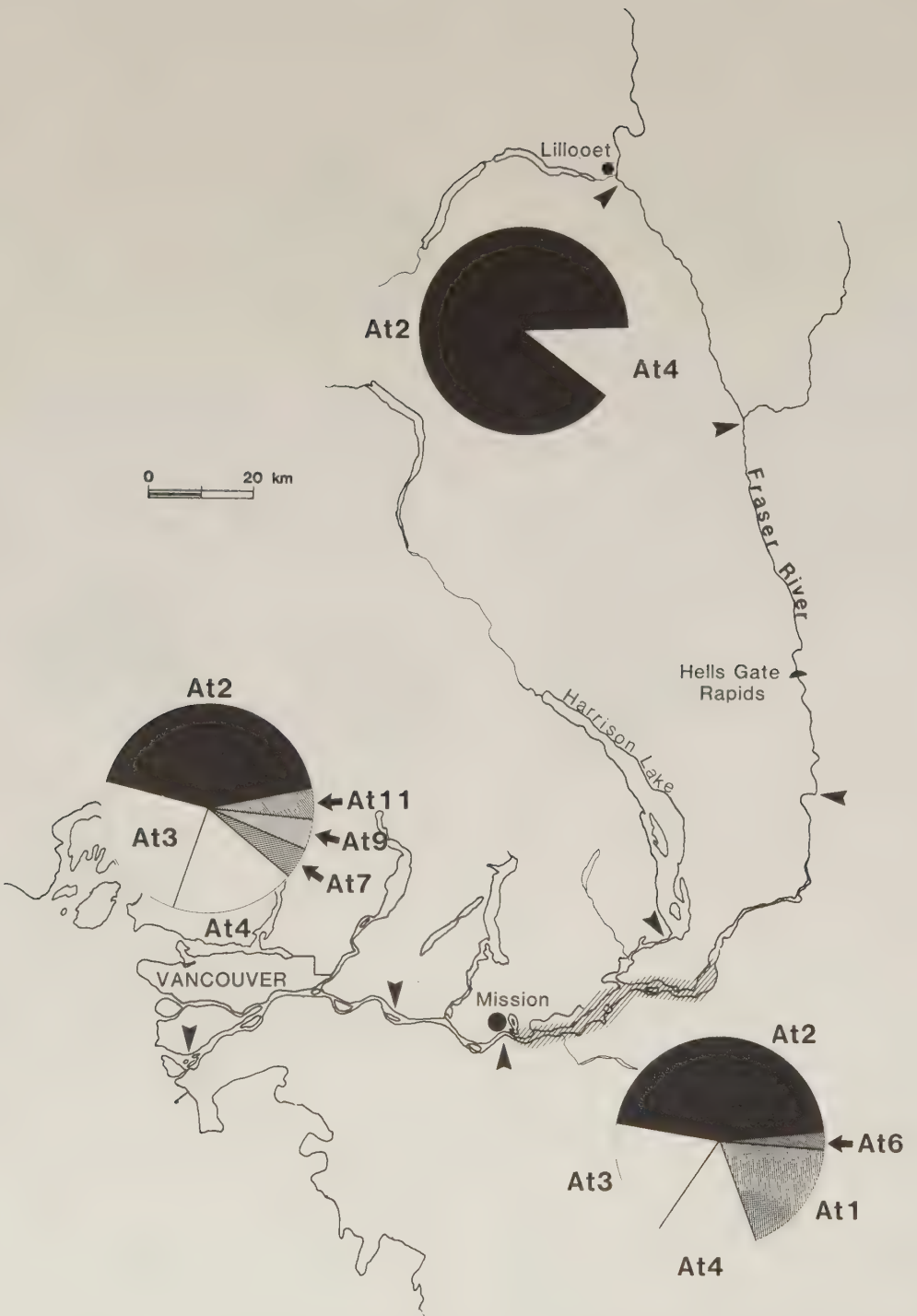

FIG. 5. MtDNA genotypic frequencies in the Fraser River. Arrows indicate the edges of sample areas for the lower (n = 22), middle (n = 74), and upper (n = 9) river. Shaded area upstream of Mission City covers the range of genotype At1.

ulations (h = 0.90 ± 0.06) of a closely related species, *C. lavaretus* (Bernatchez et al. 1989). Genetic bottleneck effects were probably less severe for the Eurasian species because over its contemporary range, glaciation was incomplete and several local refugiums probably existed. Ashley and Wills (1987) concluded that, in the deer mouse (*Peromyscus maniculatus*), a few recent colonization events were responsible for lower mtDNA heterogeneity of island populations (h = 0.0–0.44) compared with mainland groups (h = 0.67–1.0).

The *A. transmontanus* population that colonized the Fraser River (h = 0.70) had considerably higher mtDNA diversity than the putative founder population of the Columbia River (h = 0.36), contrary to expectation. Further, the upper Columbia River population (h = 0.30) had reduced diversity relative to the lower Columbia River population (h = 0.49). Log-likelihood ratio chi-square tests confirmed genotypic frequencies were significantly different in all comparisons between the Fraser River, the lower Columbia River, and the upper Columbia River.

TABLE 3. Estimates of mtDNA diversity (h) and nucleotide diversity (π) using the methods of Nei and Tajima (1981) and Nei and Li (1979), respectively.

Location	n	h	π
Fraser and Columbia rivers	178	0.65	0.135
Fraser River	105	0.70	0.157
1987	66	0.75	0.151
1988	19	0.53	0.134
1989	20	0.51	0.142
Columbia River	73	0.36	0.076
Lower	19	0.49	0.103
Upper	54	0.30	0.067
Bonneville	25	0.16	0.039
The Dalles	24	0.42	0.096
John Day	5	0.40	0.029

It is unlikely that additional samples would have increased the detection of novel mtDNA genotypes in either river system (given the enzymes used in this study). The combinatorial analysis estimated that a minimum sample size of 33–39 individuals is sufficient to fully detect mtDNA diversity in both river systems (Fig. 6). Samples for the Columbia (n = 73) and Fraser (n = 105) rivers were considerably larger than this estimate. The consistently fewer number of genotypes detected over a range of sample sizes in the Columbia River, relative to the Fraser River, is further evidence of lower genetic diversity in the Columbia River.

The mtDNA diversity of both river samples combined (h = 0.65) is near the lower estimates for natural populations of Atlantic salmon (*Salmo salar*) (h = 0.60) and brown trout (*Salmo trutta*) (h = 0.70–1.0; Gyllensten and Wilson 1987). In these same salmonids, mtDNA diversity levels determined for hatchery populations (h = 0.0–0.02) founded by small maternal groups approached the diversity estimate of the entire upper Columbia River sturgeon population (h = 0.30) and, in particular, the Bonneville reservoir sample (h = 0.17).

There are two possible explanations for higher mtDNA diversity in sturgeon populations of the recolonized Fraser River relative to the Columbia River refugium. First, migrants from another southern population may have significantly added to the Fraser River mtDNA gene pool. However, with the exception of the Sacramento River, other Pacific Coast rivers do not support substantial sturgeon populations. The single individual from the Sacramento River examined had a unique, but closely related genotype, not found in either of the more northerly rivers.

The second, and more plausible, explanation is that although the Columbia River was the source of present mtDNA lineages in the Fraser River, postglacial events have lowered mtDNA diversity in the Columbia River population. Recent overexploitation and habitat destruction could have greatly reduced genetic diversity in sturgeon. Semakula and Larkin (1968) documented a precipitous decline in the commercial catch of sturgeon on the Fraser River from over 2 500 000 kg in 1897 to below 44 000 kg by 1916. Columbia River stocks suffered a parallel decline over the same time period (Galbreath 1985).

Habitat changes have occurred in both river systems but have been more extensive in the Columbia River basin. Hydroelectric dams built on the Columbia River in the 1930's and 1950's have probably caused major alterations in sturgeon migration patterns and spawning areas. The abrupt appearance of these barriers over a period of only a few years may have been responsible for the observed segregation of certain genotypes in areas either upstream or downstream of the Bonneville Dam.

In 1912, an accidental rock slide blocked the Fraser River at Hell's Gate, about 200 km from the mouth. mtDNA diversity was reduced in the sample collected above Hell's Gate (h = 0.22) relative to the lower Fraser River (h = 0.72). This intrariver difference in diversity estimates is similar to that observed in the Columbia River between sturgeon populations located above and below the Bonneville Dam.

Similar patterns of lower genetic diversity in recently isolated, freshwater populations have been observed in other fish species. A freshwater *S. trutta* population trapped above a waterfall had lower heterozygosity at protein loci compared with an anadromous

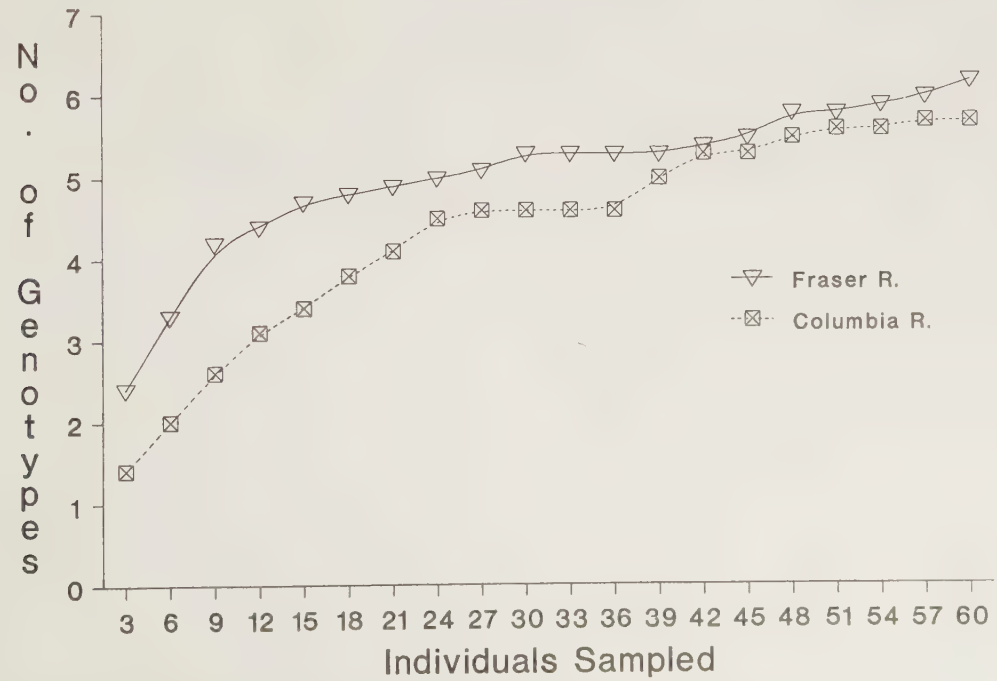

FIG. 6. Sample size and detection of *A. transmontanus* genotypes in the Fraser and Columbia rivers. Each data point represents the mean of 10 random subsamples.

population found in the same river basin (Hindar et al. 1991). Genetic differentiation between landlocked and anadromous forms was significantly greater than that between sympatric *S. trutta* populations with different life histories traits. Australian populations of *Galaxias ruttaceus* landlocked in lakes had lower mtDNA diversity than stream populations with access to estuaries and the sea (Ovenden and White 1990). The recent formation of these Australian lakes (3000–7000 yr ago) was suggested as a severe but transitory bottleneck which reduced mtDNA diversity.

There is no apparent divergence between mtDNA genotypes found above Bonneville Dam and those occurring in the lower Columbia River and the Fraser River. The probability of detecting nucleotide changes so soon after the occurrence of a genetic bottleneck is extremely low (Wilson et al. 1985). However, a steep reduction in mtDNA or genetic diversity has occurred in the landlocked sturgeon population.

Genetic drift in isolated demes is unlikely to have caused a reduction in mtDNA diversity in upper Columbia River sturgeon populations because fewer than four consecutive generations have passed since the closure of the Bonneville Dam. Lower mtDNA diversity in the upper Columbia River is most probably the result of selective pressures for individuals which can reproduce in the landlocked, freshwater habitat. This is not to suggest that selection is acting at the mtDNA level, but that mtDNA genotypes are neutral markers associated with surviving maternal lineages.

Human pressures upon populations can be highly selective and result in a net reduction in genetic diversity (Nelson and Soulé 1987). Dams on the Columbia River may have reduced sturgeon spawning success by denying lower river sturgeon access to upstream spawning grounds. Upstream sturgeon populations are similarly excluded from estuaries that have greater food productivity. Also the dams have greatly altered natural current velocities that may be critical to sturgeon spawning success (Doroshov 1985; Brannon et al. 1985).

If particular groups of females with synchronous migration patterns are distinguishable by mtDNA genotype, the present-day distribution of mtDNA genotypes could be indicative of the location of these maternal cohorts when the dams were closed. The presence of two genotypes (At3 and At6) in both the Fraser and upper Columbia rivers, and their absence from the lower Columbia River, proves that upper Columbia sturgeon once migrated throughout the basin and into marine coastal waters.

Any reduction in genetic diversity in sturgeon populations should be of special concern in the conservation of this species given its long generation time and advanced age of reproductive maturity. The endangered or threatened status of two thirds of the North American sturgeon indicates that this ancient group has not fared well in recent times (Williams et al. 1989).

Previous evolutionary studies of intraspecific mtDNA variation have been mainly concerned with the phylogeographical relationship of mtDNA genotypes as a reflection of the influence of natural geographical events on population structure. We suggest that human intervention in animal populations can also produce detectable shifts in mtDNA genotypic frequencies over a relatively short time.

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Appendix A

TABLE A.1. DNA fragment lengths (in base pairs) for the restriction enzymes used in this study. Species are *A. transmontanus* (A.t.) and *A. medirostris* (A.m.). Intraspecific polymorphic DNA fragment patterns are denoted by the letter codes (A, B, or C) used in Table 2. Variable length fragments known to span the control region are indicated by an asterisk.

*Apa*I		*Eco*RI		*Hind*III		*Bgl*II		*Pvu*II		*Xba*I	
A.T.	A.m.	A.t.	A.m.	A.t.	A.m.	A.t.	A.m.	A.t.	A.m.	A.t.	A.m.
7200	7200	8400	8600	12 000	12 000	16 500	16 500	16 500	10 000	11 000	8200
5400	5400	8200	7600	3 000	3 000				6 400	4 700	4700
											2800
4300	4300			1 100	1 100					1 100	1100

*Acc*I			*Hae*II		*Ava*II			*Bcl*I		
A.t.-A	A.t.-B	A.m.	A.t.	A.m.	A.t.-A	A.t.-B	A.m.	A.t.-A	A.t.-B	A.m.
4600*	4600*	4600*	15 000	15 000	6200	4000	8300*	8000	8000	4800
3600	2800	2500	1 800*	1 800*	2700*	2700*	1700	2500	2500	4800*
1900	1900	1900			2100	2200	1600	2300	2300	2500
1750	1750	1750			1400	2100	1100	1800*	1370	2300
1550	1550	1550			1000	1400	1000	750	900*	750
1300	1300	1300			760	1000	760	680	820	680
1100	1100	1100			620	760	620			
560	800	1100			560	620	560			
500	560	560			520	560	520			
	500	500			400	520	400			
					340	400	340			
						340				

*Ava*I				*Hinc*II		
A.t.-A	A.t.-B	A.t.-C	A.m.	A.t.-A	A.t.-B	A.m.
6600	11 000	11 000	6600	3700	5000*	5000*
5400	5 400	3 400	5400	2700	3700	3700
4600		2 000	4600	2500	2700	2700
				2500*	1400	1400
				1400	1400	1100
				1400	1100	940
				1100	580	580
				580	450	480
				450		450

Lipid Condition and Survival in Shrimp (*Pandalus borealis*) Larvae

Patrick Ouellet[1] and Christopher T. Taggart

Department of Oceanography, Dalhousie University, Halifax, N.S. B3H 4J1, Canada

and Kenneth T. Frank

Department of Fisheries and Oceans, Marine Fish Division, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, N.S. B2Y 4A2, Canada

Ouellet, P., C. T. Taggart, and K. T. Frank. 1992. Lipid condition and survival in shrimp (*Pandalus borealis*) larvae. Can. J. Fish. Aquat. Sci. 49: 368–378.

Groups of shrimp (*Pandalus borealis*) larvae were reared under different food concentrations and types to assess changes in condition and survival during development. As expected, rate of growth (wet weight) was higher for larvae fed on 300 *Artemia* nauplii·L^{-1} (San Francisco strain: 0.0528 mg·d^{-1}; Canada strain: 0.0355 mg·d^{-1}) compared with those reared at 150 nauplii·L^{-1} (San Francisco strain: 0.0211 mg·d^{-1}), larvae fed *Isochrysis* cells only, and no food. Shrimp larvae reared without food and larvae fed on *Isochrysis* cells showed a decreasing trend in wet weight during development and did not survive beyond stage II. The triacylglycerol (TAG) content of larvae accumulated rapidly during the initial phase of intermoult followed by a decline to a minimum coincident with ecdysis. High mortality (average 2.2%·d^{-1}) occurred during the first phases of development (first 25 d) for all rations. Differences among experiments in the magnitude of the mortality (from 0.86 to 4.66%·d^{-1}) at the first moult were related to the proportion of larvae in poor TAG condition. The results are consistent with the concept that the TAG condition index can be used to forecast, on a relative basis, differential survival among larval groups.

Des groupes de larves de crevette (*Pandalus borealis*) ont été gardés sous différent types et concentrations de nourriture, afin d'étudier l'évolution de la condition et de la survie au cours du développement. Des différences dans la croissance des larves ont été observées tel que prévues à partir des différences de qualité des diètes. Les plus forts taux de croissance (poids humide) ont été observés pour les larves nourries à une concentration de 300 nauplii d'*Artemia*·L^{-1} (souche San Francisco: 0,0528 mg·d^{-1}; souche Canada: 0,0355 mg·d^{-1}), relativement aux larves nourries à 150 nauplii·L^{-1} (souche San Francisco: 0,0211 mg·d^{-1}), aux larves nourries d'algues *Isochrysis* et aux larves gardées sans nourriture. Les larves de crevette gardées sans nourriture et les larves nourries de cellules d'*Isochrysis* ont montré une décroissance en poids au cours du développement et n'ont pas survécu après la première mue (début du stade-II). Le contenu en triacylglycerol (TAG) des larves montre une rapide accumulation de réserves énergétiques tôt pendant l'intermue, suivie d'une diminution jusqu'à un minimum coïncident avec les mues. Des mortalités importantes (moyenne de 2,2%·d^{-1}) sont survenues au cours des premières phases du développement (premier 25 jours) dans chaque diète. Les différences dans l'amplitude des mortalités (0.86 à 4,66%·d^{-1}) pendant la première mue entre les expériences étaient reliées à la proportion de larves en pauvre condition. Les résultats supportent l'idée qu'un indice de la condition larvaire peut être utiliser pour prédire, sur une base relative, la survie des groupes de larves.

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Much of the current research on the causes of recruitment variability in marine populations is built on the hypothesis that recruitment is a function of larval abundance and survival. However, several attempts to correlate recruitment indices with environmental variables supposedly influencing survival of larvae have failed, and it has been suggested that year-class strength is not generally correlated with larval abundance (Sissenwine 1984). Reasons for the lack of correlation may be inadequate and inaccurate abundance estimates and/or that the environmental variables used did not directly influence survival of the larval stage. One way to strengthen the correlative approach is to better define the factors of the environment directly accountable for differential survival in larval populations. A second is to adjust the larval abundance estimates with expectations of differential survival that are based on the condition of the larvae.

Several methods have been proposed to assess condition of larval fish based on morphometric and histological indexes (Shelbourne 1957; Theilacker 1978; O'Connell 1976). However, these methods have the disadvantage of relying on some subjective categories, are time consuming to derive, and can be difficult to use. Ehrlich (1974) criticized the use of a morphometric condition index and proposed the use of elemental chemical composition to assess condition. More recently the determination of total RNA content and the RNA/DNA ratio have been employed to assess the growth of fish larvae in the sea (Buckley 1984; Buckley and Lough 1987; Robinson and Ware 1988). A biochemical condition index based on the energetic (i.e. lipid) content of individual larvae has also been recently proposed (Fraser et al. 1987; Fraser 1989; Hakanson 1989a, 1989b). However, the quantitative relationship between any condition index and survival probability remains to be

[1]Permanent address: Ministère des Pêches et des Océans, Institut Maurice-Lamontagne, Ste-Flavie (Québec) G5H 3Z4, Canada.

established. If established, a powerful means of improving recruitment studies will be available.

Lipids, and particularly neutral lipids, have been identified as the main energetic reserve for the larvae of many invertebrate marine species (oyster, Holland and Spencer 1973; scallop, Whyte et al. 1987; lobster, Sasaki et al. 1986). The pelagic development of decapod crustacean larvae entails a sequence of moult and intermoult periods during which the feeding regime appears to be the determinant of success (Anger and Dawirs 1981; Gore 1985). In this study, we assessed the condition (lipid content) and survival among groups of shrimp (*Pandalus borealis*) larvae reared in a variety of feeding regimes. Our objective was to evaluate the possibility that survival and physiological condition can be quantitatively linked in a predictive manner.

As the lipid content of individual shrimp larvae and its relationship to feeding history have not been reported, we designed this initial study with several objectives: (1) to measure the lipid content of individual larvae, (2) to measure changes in lipid content in response to different foods, and (3) to assess the relationship between lipid content and survival. This paper presents results that demonstrate the potential use of variability in larval condition as a predictor of differential survival among larval populations.

Materials and Methods

Lipids and Larval Development

A key assumption in our approach is that the pattern of physiological and biochemical changes apparent in crab (*Hyas araneus*) larvae (Anger and Dawirs 1981; Anger et al. 1989) is applicable to shrimp. Stated briefly, early in the moult cycle, postmoult period, water and minerals are taken up by the larvae. This is followed during the intermoult by the accumulation of organic reserves and tissue growth when food is available. Under ideal conditions, the postmoult and intermoult are assumed to last for approximately one third of the stage duration (Anger and Dawirs 1981). From the time accumulated reserves reach saturation levels (point of reserves saturation (PRS)), moulting hormones (β-ecdysone) ensure the completion of the moult cycle (independent of further feeding). These two phases are reflected by changes in the biochemical composition (C/N ratio) of the larvae showing lipid (carbon (C)) accumulation during the first part of each moult cycle and protein accumulation (nitrogen (N)) during the premoult stages (Anger et al. 1989). The results of simple simulation are presented in Appendix 1 to support the assumption.

Feeding Experiments

Ovigerous female shrimp were obtained from the northern Gulf of St. Lawrence in mid-April 1989 and were transferred to the Aquatron Laboratory at Dalhousie University, Halifax, Nova Scotia (Canada), where they were incubated, in the dark, in a 80-L container of recirculating seawater (~4°C). Hatching began 2 d later. When a sufficient number of larvae (>50) were observed in the incubator, groups of 50–400 larvae were isolated for use in the various feeding experiments. Actively swimming larvae were collected with a wide-aperture pipette and were transferred to rearing tanks filled with filtered (15 μm) seawater. As collections relied on the hatching rate, there were different initial numbers of larvae among the different experiments. The rearing tanks were chilled using Aquatron seawater.

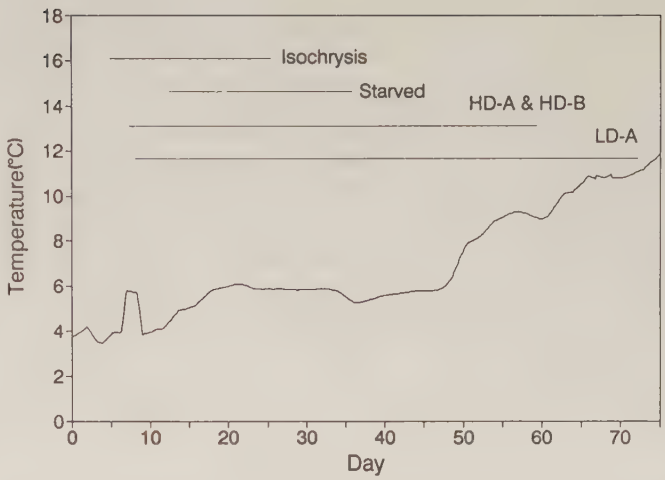

FIG. 1. Hourly average of water temperature for the duration of the rearing. Horizontal bars indicate the relative position of each experiment over the period.

This method maintained the rearing temperature at an average of 5.4°C (SD 0.82) during the first 50 d of the experiment (Fig. 1). As the limited temperature variation was synchronous among all containers, it was not considered a factor in the experimental design.

The choice of foods for the larvae was determined by the previous studies describing successful development of shrimp larvae fed *Artemia* nauplii alone e. g. *P. borealis* (Stickney and Perkins 1981; Wienberg 1982; Nunes 1984), *Palaemon serratus* (Wickins 1972), and *Palaemonetes* spp. (Knowlton 1974; Freeman 1990). Food of varying quality was purposely selected by using different *Artemia* strains (Wickins 1972; McConaugha 1985) and feeding the larvae the small flagellate *Isochrysis* spp. (Nunes 1984; Harms and Seeger 1989).

The effect of different foods on larval condition and survival was assayed by feeding two groups of 100 recently hatched (<2 d old) shrimp larvae a ration of *Artemia* nauplii ($300 \cdot L^{-1} \cdot d^{-1}$) newly hatched from (a) San Francisco Bay strain cysts (high density A (HD-A)) and (b) Canada strain cysts (high density B (HD-B)). The larvae were held in 5-L Erlenmyer flasks with a constant air stream to ensure oxygenation and the uniform dispersion of nauplii. The containers were cleaned and the water changed every second day for the duration of the experiment. The static container experiment was completed by providing a group of 50 larvae contained in a 3-L Erlenmyer flask a concentration of the algae *Isochrysis* spp. (2×10^4 cells$\cdot L^{-1} \cdot d^{-1}$) and treated as above.

A second treatment involved feeding a group of 400 shrimp larvae in a 40-L flow-through reservoir 150 nauplii$\cdot L^{-1} \cdot d^{-1}$ of the San Francisco strain *Artemia*. In comparison with the HD-A ration, this treatment served as a low density A (LD-A) ration. Finally, a group of 200 shrimp larvae was held without food (Starved) in a similar flow-through reservoir to serve as control for the biochemical analysis.

A modification of all the nauplii feeding experiments was applied two thirds of the way through the rearing period (day 30) by reducing the food ration to one half. This modification was designed to increase the nutritional stress on the larvae during a period of constant survival after the first two moult cycles and to examine the short-term response (lipid content) of the larvae to a change in prey concentration. The ratios were returned to the initial levels after the pseudo-fasting period of

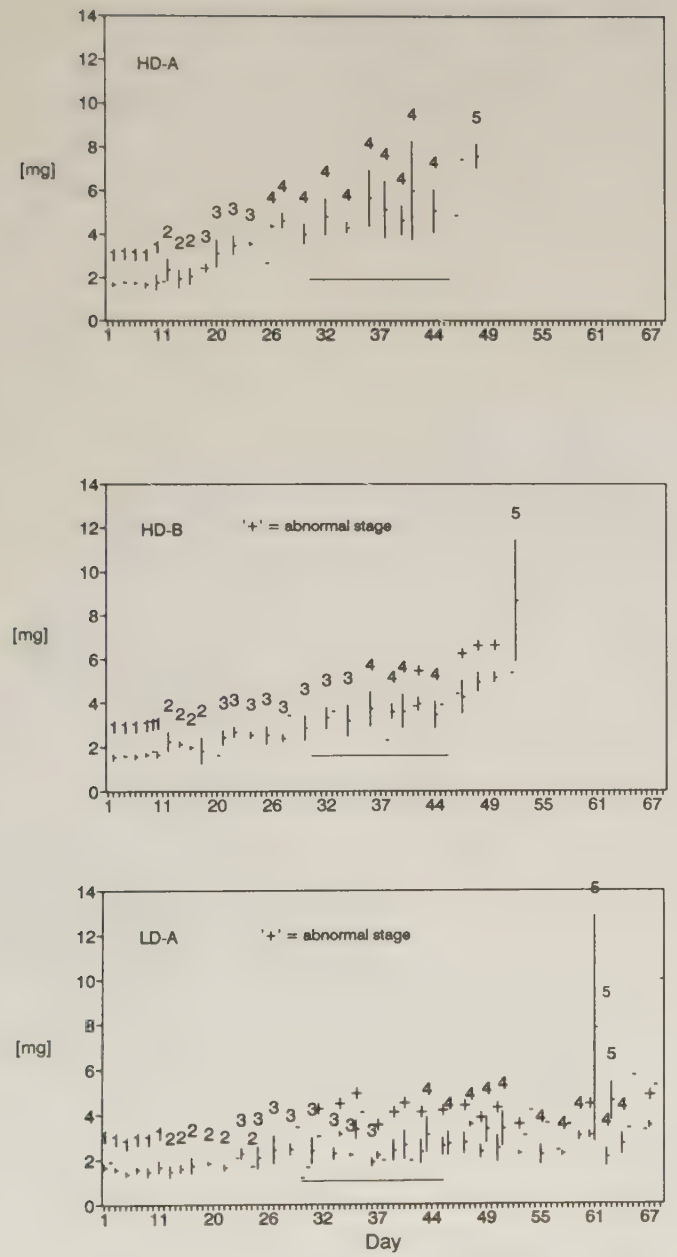

FIG. 2. Average wet weight (±1 SD) of live shrimp larvae sampled for biochemical analysis from the *Artemia*-fed groups two strains of *Artemia* at high density (HD-A, HD-B) and one at low density (LD-A)). The numerals and symbols above each data point indicate the developmental stage. The horizontal line represents the period of half food ration.

15 d (day 45). The maximum larval density never exceeded 1 larva·50 mL^{-1} in any reservoir to minimize the chances of cannibalism (Nunes 1984).

Dead shrimp larvae from each container were counted and preserved each day. Live larvae (three for each of the HD-A, HD-B, and *Isochrysis* rations and five from the LD-A and Starved larvae) were collected every second day for biochemical analysis. All larvae (dead and live) were staged, measured (total and carapace length), wet-weighed after blotting on absorbent paper, and immediately stored individually in liquid nitrogen. Staging of the larvae was based on Haynes (1979). Atypical stages of development after a given moult were defined as abnormal stages.

Biochemical Analysis

The extraction procedure for the neutral lipid was based on the protocols of Gardner et al. (1985) and Fraser et al. (1985). No more than two larvae, i.e. for the smallest zoea I, and usually only one was homogenized with a glass pestle in 500 μL of solvent (dichloromethane–methanol (2:1, v/v)) in an ice bath. The homogenate was rinsed with 250 μL of a KCl solution (0.88%) and centrifuged twice at 4000 rpm for 3 min. The lipid fraction was removed after each wash and transferred to a clean tube. A known quantity (10–20 μg) of Nonadecane was then added for use as an internal standard. The solvent was evaporated under a nitrogen stream and the lipid was resuspended in 15–20 μL of solvent before the entire sample was spotted on a SIII-Chromarod for quantification using flame ionization detection with a model MK-III IATROSCAN (Iatron, Inc., Japan). The rods were initially developed for 30 min in hexane – ethyl ether – formic acid (82:2.5:0.045, v/v/v) allowing separation of the Nonadecane and the esters. Each rod was secondarily developed for 30 min in hexane – ethyl ether – formic acid (55:29.7:0.075, v/v/v) for separation of the triacylglycerol (TAG), free fatty acid (FFA), and sterol constituents. The entire procedure took ~3 h, and 20–30 larvae could be analyzed by a qualified technician within a regular workday. The quantification of each constituent was based on calibration curves constructed from solutions of standard neutral lipids (Parrish and Ackman 1985; Fraser et al. 1985).

TAG is the primary storage form (as fat) of organic energy in animals, and so the TAG to wet weight ratio ((TAG mg/WW mg)·1000)) was used as the index of larval feeding condition. The amount of FFA in a sample is an indicator of the quality of preservation and handling (Christie 1982). A large quantity of FFA indicates that the sample suffered degradation before, during, or after the storage period. Quality control was based on the arbitrary rule that when the quantity of FFA exceeded the quantity of TAG the sample (8% overall) was not included in the analyses.

Data Analysis

Life table techniques were used to characterize the survival pattern in the larval groups and to compare the results among the different treatments. The method can accommodate censored data, i.e. the removal of live larvae for biochemical analysis which cannot be counted as dead in the survival estimates (Cox and Oakes 1984; Chambers and Leggett 1989). The life table data were used to estimate (1) the Survival function (i.e. the cumulative probability of surviving (event T) beyond a given time t),

$$S_{(t)} = \text{Prob}(T > t)$$

$$\hat{S}_{(t)} = \prod_{t=0}^{k} \left(1 - \frac{d_t}{N_t}\right)$$

where d_t is the number of dead larvae for the interval k (t, $t + \Delta t$) and N_t is the initial number of live larvae at the beginning of the interval, corrected for larvae removed for the biochemical analysis, and (2) the Hazard function (i.e. the conditional probability of death in a given interval),

$$h_{(t)} = \lim_{\Delta t > 0} \frac{\text{Prob}\{(t < T \leq t + \Delta t | T > t)\}}{\Delta t}$$

TABLE 1. Mean total length (TL) and carapace length (CL) (millimetres) and standard deviation for each larval stage and treatment. Values in parentheses represent relative (percent) length increase between moults (MI = (postmoult CL − premoult CL)/premoult CL).

Stage	HD-A TL ± SD CL ± SD *n* [MI]	HD-B TL ± SD CL ± SD *n* [MI]	LD-A TL ± SD CL ± SD *n* [MI]	*Isochrysis*-fed TL ± SD CL ± SD *n* [MI]	Starved TL ± SD CL ± SD *n* [MI]
I	6.58 ± 0.23 1.476 ± 0.086 16 (—)	6.537 ± 0.24 1.427 ± 0.074 17 (—)	6.255 ± 1.18 1.401 ± 0.083 44 (—)	6.288 ± 0.22 1.307 ± 0.085 27 (—)	6.335 ± 0.26 1.375 ± 0.096 27 (—)
II	6.886 ± 0.45 1.647 ± 0.091 11 (11.5)	6.873 ± 0.24 1.6 ± 0.098 14 (12.1)	6.536 ± 0.31 1.571 ± 0.099 27 (12.1)	6.158 ± 0.17 1.395 ± 0.201 4 (6.7)	6.431 ± 0.29 1.472 ± 0.08 15 (7.0)
III	8.185 ± 0.54 1.837 ± 0.24 12 (11.5)	7.686 ± 0.433 1.763 ± 0.159 23 (10.2)	7.148 ± 0.46 1.723 ± 0.173 31 (9.7)		
III+			7.732 ± 0.39 1.481 ± 0.109 42 (5.3)		
IV	9.409 ± 0.58 2.165 ± 0.215 27 (17.8)	8.79 ± 0.49 2.061 ± 0.178 14 (16.9)	7.999 ± 0.62 1.956 ± 0.134 27 (7.8)		
IV+		9.652 ± 0.23 2.187 ± 0.138 9 (6.1)	8.02 ± 0.84 2.044 ± 0.152 10 (4.5)		
V	9.852 ± 0.35 2.134 ± 0.071 5 (6.9)	10.08 ± 0.31 2.78 ± 0.245 3 (27.1)	10.13 ± 1.16 2.17 ± 0.206 5 (5.9)		

or, following conditional probability theory,

$$\text{Prob}\{(t<T \leq t + \Delta t)\} = f_{(t)} \cong -\frac{d\hat{S}_{(t)}}{dt}$$

$$\hat{h}_{(t)} = \frac{\hat{f}_{(t)}}{\hat{S}_{(t)}} = -\frac{d[\ln \hat{S}_{(t)}]}{dt}$$

$$\hat{H}_{(t)} = -\ln(\hat{S}_{(t)}).$$

The two functions are interrelated and the general expression for the Survival function in terms of the Hazard function is $S_{(t)} = \exp[-H_t]$. No attempt was made to fit distribution models to the data. However, the relationship between $\ln[-\ln(S_{(t)})]$, i.e. $\ln(H_{(t)})$, and ln(Day) for the periods of high mortality in each experiment was linear and conformed to the Weibull distribution (Lawless 1982), i.e. the hazard function is a power function of time:

$$S_{(t)} = \exp[-\lambda(\text{Day})^{\beta}].$$

Estimates of the parameters (λ, β) were obtained by linear regression and were used to compare the distributions among the experiments.

Results

Larval Growth and Development

The different feeding conditions were reflected in the marked differences in growth (wet weight) and development (stage) of shrimp larvae (Fig. 2). Limited or insignificant increases in size and length (Table 1) were observed from stage I to stage II zoeae, but different growth rates were observed from stage II to stage V larvae among the feeding regimes (Table 1; Fig. 2). The rate of growth was significantly higher (0.0528 and 0.0355 $mg \cdot d^{-1}$) for HD-A and HD-B larvae relative to the LD-A larvae (0.0211 $mg \cdot d^{-1}$). The *Isochrysis*-fed and Starved larvae did not survive past the first moult (early stage II) and the larvae in both groups showed a decrease in wet weight during development (Fig. 3A).

The larvae that grew faster also developed and moulted more rapidly (Table 2). Development was slower for the larvae on the HD-B ration and abnormal stages were observed before reaching a distinct stage V (Fig. 2). Development was more variable in larvae reared on the LD-A ration. Abnormal stages were observed cooccurring with distinct stage IV and V larvae (Fig. 2; Table 2). However, despite the relatively slow growth, moulting continued regularly until the end of the experiment. The first moult (I to II) was delayed by 2–3 d in the *Isochrysis*-fed, Starved, and LD-A larvae relative to the HD-A and HD-B larvae (Table 2).

Differences in average wet weight at a given stage were observed between the dead and live larvae within the treatments (Fig. 4). With the exception of the Starved group (where live larvae can not assimilate any food and growth), dead larvae were significantly smaller (by 10–30%) in size. The degradation of the biochemical material prevented a comprehensive analysis of the lipid content of the dead shrimp larvae.

Neutral Lipids and Development

The standardized index of larval condition (TAG wet weight ratio (TAG/WW)) showed considerable variation during larval development (Fig. 3B, and 5). At hatching the index was at or near zero in all treatments. The index fluctuated with no distinct periodicity and never exceeded 0.7 in the *Isochrysis*-fed and

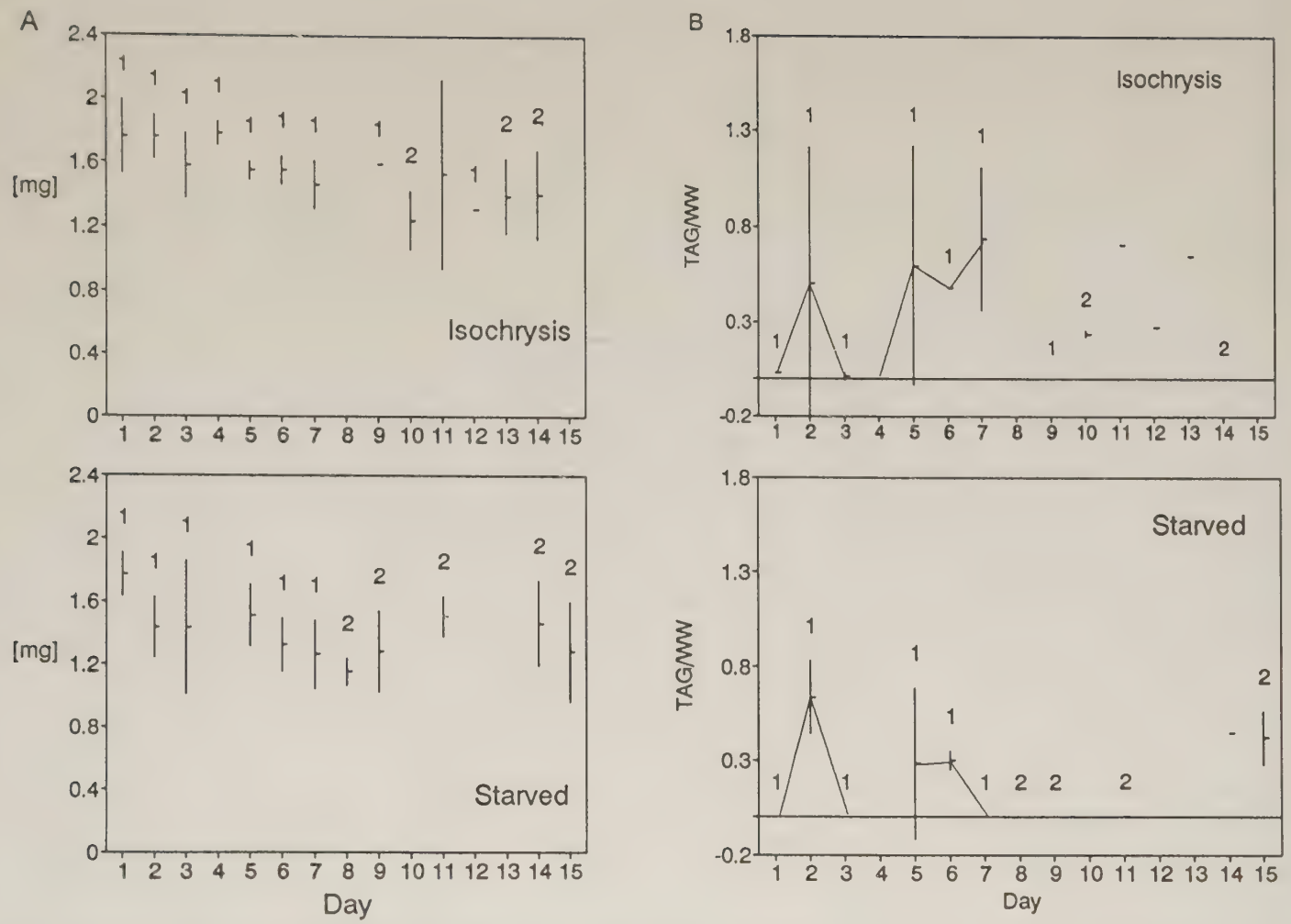

FIG. 3. (A) Average wet weight (± 1 SD) of shrimp larvae sampled live for biochemical analysis from the *Isochrysis*-fed and Starved groups. The numerals and symbols above each data point indicate the development stage. (B) Average TAG/WW ratios (± 1 SD) of the shrimp larvae sampled live in the *Isochrysis*-fed and Starved groups.

Starved groups (Fig. 3B). In contrast, in all the *Artemia* nauplii groups the index began increasing immediately after hatching, reaching a maximum 3–5 d later (Fig. 5). The TAG/WW ratio then decreased to a minimum that coincided with moulting. The pattern was observed for the LD-A through the third moult and for the first two moult cycles in HD-B larvae (Fig. 5). In contrast, in the HD-A larvae the condition index reached a maximum 5 d after hatching, but no minimum coincident with the first moult was observed (Fig. 5). Finally, lower values of the TAG/WW ratios were observed during the period of half-food rations in each treatment (Fig. 5).

The frequency distribution of individual condition (TAG/WW) reflected the effect of the different rearing conditions on the overall condition of the larval groups (Fig. 6). The estimates were made on individual larvae only. The majority (>50%) of *Isochrysis*-fed and Starved larvae had a very low (≤0.2) condition index. In comparison, the *Artemia*-fed groups had a majority (≥70%) of stage I and II (live) larvae with an index of >0.2 (mean = 0.508 (CV = 58.3%), 0.682 (84.2%), and 0.604 (114.2%) for HD-A, HD-B, and LD-A, respectively). The proportion of larvae (stages I and II) with a lower index (TAG/WW ≤ 0.2) was higher in the LD-A larvae relative to the HD-A and HD-B larvae (fig. 6), but Kolmogorov–Smirnorv tests revealed no significant differences in the frequency distributions between the *Artemia*-fed groups when tested pairwise ($P > 0.05$).

Survival

Larval survival for all treatments decreased after 10 d (first moult) and, not surprisingly, declined more rapidly in the *Isochrysis*-fed and Starved larval groups (Fig. 7A). After the first moult, survival remained relatively constant in the *Artemia*-fed groups (HD-A, HD-B), except at 60 d in the LD-A larvae when survival decreased from 0.6 to 0.2 in a period of 5 d. The event coincided with increasing water temperature, above 8°C, that could have affected respiratory metabolism and survival. Mortality was not random and was more likely to occur during moulting events (Run test, $0.01 < P < 0.06$). Mortality was especially concentrated during and following (>10 d) the first moult as shown from the Hazard functions (Fig. 7B). Increasing risk of dying, i.e. higher slope of the cumulative Hazard function, corresponded to the different feeding regimes (Table 3) and were well correlated with the condition indices (proportion of larvae in TAG/WW ≤ 0.2) of the larval groups (Fig. 8).

TABLE 2. Moult frequency and developmental stage duration (days) of shrimp larvae in each treatment. Min. = number of days since the beginning of the experiment for the first observation of the stage; Max. = number of days for completion of the stage (e.g. all larvae have moulted to the next stage); Mean = mean duration of the stage.

	Moult sequence					
From stage:	I	II	III	III (III+)	IV	IV (IV+)
To stage:	II	III	III+	IV	IV+	V
HD-A						
Min.	10	18		25		46
Max.	12	20		28		END
Mean	11	8		7.5		20
HD-B						
Min.	10	19		28	36	45
Max.	13	22		34	38	END
Mean	11.5	9		10.5	6	11.5
LD-A						
Min.	13	22	30	43	61	65
Max.	16	27	35	55	END	END
Mean	14.5	10	8	16.5	15	5
Isochrysis-fed						
Min.	13					
Max.	16					
Mean	14.5					
Starved						
Min.	12					
Max.	14					
Mean	13					

Discussion

Our results support the proposed use of a relatively easily determined biochemical condition index as an indicator of survival potential among various larval groups. These are encouraging results given the difficulty in the past of establishing the full utility of trophic factors in recruitment studies (Rothschild et al. 1989).

Larval Growth and Development

The results are consistent with what is known about the pattern and sources of variation in decapod larval development (Knowlton 1974; McConaugha 1985). The different growth efficiency and development patterns observed in the larvae fed on *Artemia* nauplii rations illustrate the sensitivity of larval shrimp growth and condition to the food resources. The occurrence of abnormal stages, and the coincidence of moulting immediately following periods of pseudo-fasting (see Fig. 2), demonstrates a rapid adjustment of the larval growth pattern to changes in food quantity. The most dramatic influence of the feeding condition was observed in larvae fed the LD-A ration. These larvae displayed very limited or no growth during development, indicating that assimilated organic energy was directed toward moulting and morphogenesis at the expense of growth. However, the growth restraint was not without cost given the significantly smaller size of the dead larvae that had presumably failed to secure the minimal reserves necessary for continued survival and development.

The poor growth performance of the larvae with access only to *Isochrysis* cells corroborates earlier reports of the inadequacy of those small flagellate cells to support growth and normal development of the larvae (Nunes 1984). Survival of starved shrimp larvae through a first moult has been reported by Stickney and Perkins (1981) whereas Wienberg (1982) reported that starved larvae (kept in purified water) were all dead within 9 d and did not moult. The fact that starved larvae managed to live through the first moult in our experiment may be attributed to the relatively coarse filtering (15 μm) of the source water and the ability of larvae to absorb energy from microscopic or dissolved organic matter. We suggest that the same explanation applies to the result of Stickney and Perkins (1981) who used 25-μm-filtered seawater.

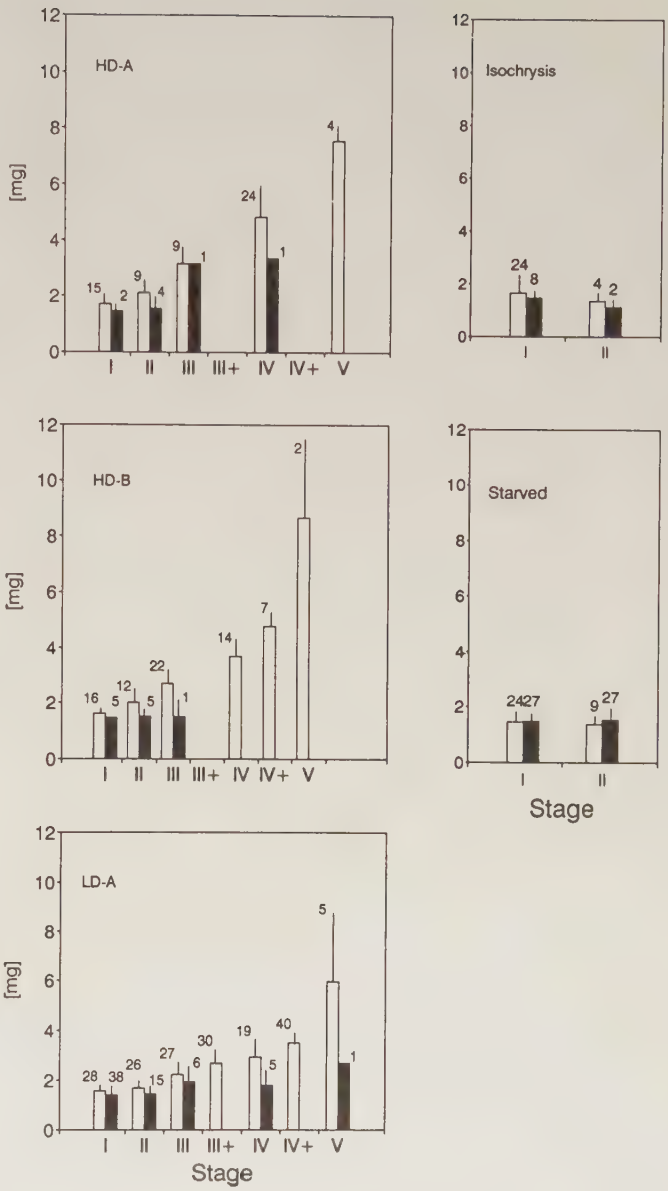

FIG. 4. Mean wet weight per stage for live (open bars) and dead (solid bars) larvae for each feeding treatment. Vertical lines represent 1 SD above the mean and the numerals indicate the number of observations for each group.

Our results must be interpreted with a clear understanding that they are based on artificial diets and rearing conditions that may cause unnatural stress to the larvae. However, the objective of the research was not to study the effect of foods on shrimp larvae development per se. By using feeding conditions of varying quality (well documented in the works cited above) to induce variability in the condition of various shrimp larval groups, it was possible to develop a quantitative index that

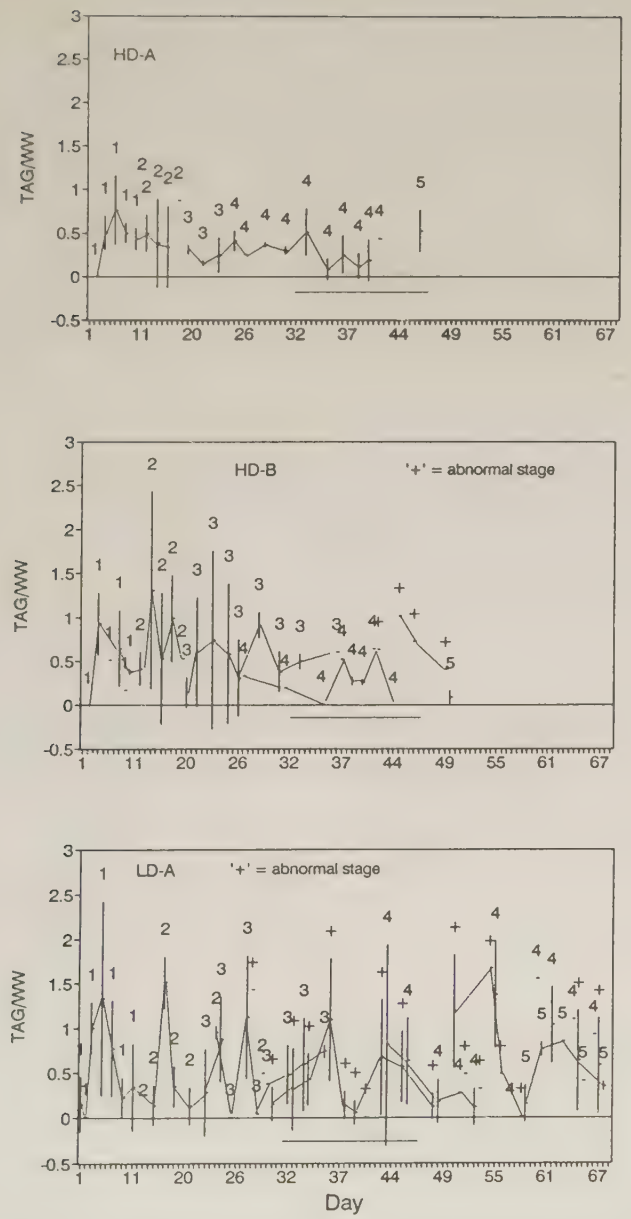

FIG. 5. Average TAG/WW ratios (±1 SD) of live shrimp larvae sampled in the *Artemia* diet treatments. The numerals and symbols above each data point indicate the larval stage. The curves track a normal (stages I through V) larval development sequence. The horizontal line indicates the duration of the half food ration.

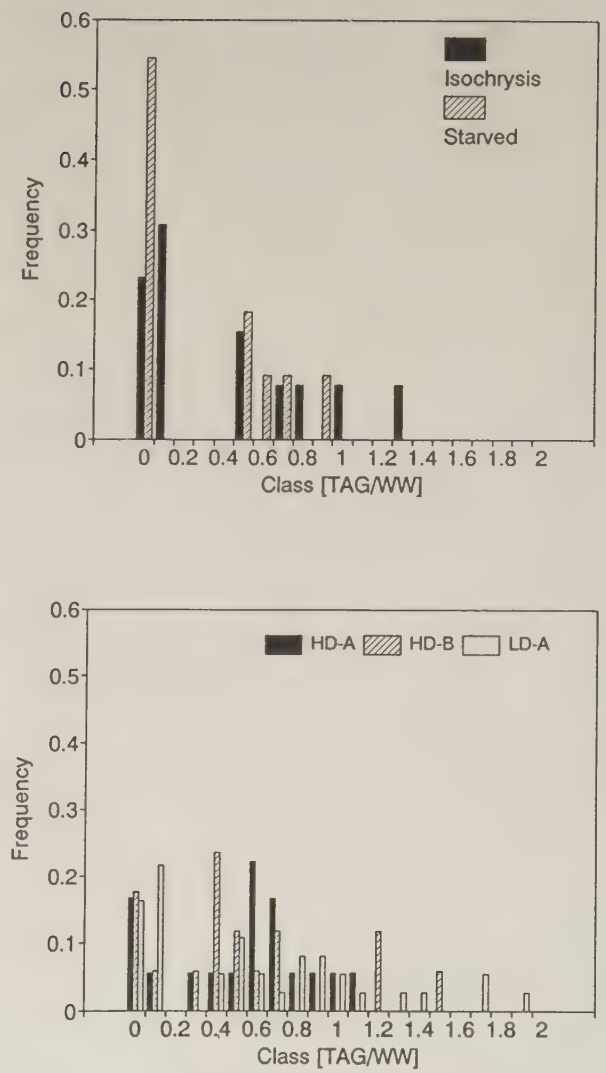

FIG. 6. Frequency (relative) distributions of individual TAG/WW ratios for all live larvae in the *Isochrysis*-fed and Starved treatments (top panel) and for live stage I and II larvae in the *Artemia* diet treatments (bottom panel).

linked larval condition and survival. Although not ideal, the feeding regimes selected are often used for rearing shrimp larvae under laboratory conditions. The quality of various *Artemia* strains used as decapod larvae food is judged, in part, on the relative proportion of long-chain polyunsaturated fatty acid (20:5ω3). In this respect the San Francisco strain (HD-A) exceeds the Canada strain (HD-B) by about 30% (McConaugha 1985, p. 134), implying that the former represents better food for the larvae. Larval growth rates were consistent with this assessment. Densities of 300 and 150 nauplii·L^{-1}·d^{-1} have been judged as adequate and marginal concentrations, respectively, in previous attempts to rear *P. borealis* larvae (Nunes 1984, although phytoplankton at 1×10^4 to 3×10^4 cells·L^{-1} was also included). Previous works on the feeding response of *P. borealis* larvae indicated that, under laboratory conditions, prey (copepodite, and copepod nauplii) densities of 80·L^{-1} were necessary for each larva to capture at least one prey item per day (Paul et al. 1979). The minimum respiratory metabolic requirement of stage I *P. borealis* larvae was estimated at 0.068 cal·ind^{-1} at 6°C, or about 8 times the estimated calorie content (0.0083 cal·ind^{-1}) of 1-d-old *Artemia salina* nauplii (Paul and Nunes 1983). Hence, high prey concentrations are necessary to insure that each shrimp larva can assimilate sufficient organic energy for survival and growth. Furthermore, as the same foods were added each day for the duration of the experiment the number of prey available to the larvae increased and paralleled the increasing metabolic demands of the larvae. There was no evidence of cannibalism in our experiment and the removal of the dead larvae minimized the possibility of necrophagy in the containers. We therefore conclude that the reuslts can provide a valuable method for identifying trophic effects on condition variation in shrimp larvae.

Lipid Content and Survival

The ecdysis cycle in shrimp larvae can be dsecribed as (a) postmoult (or posthatch for stage I zoea), (b) intermoult, (c)

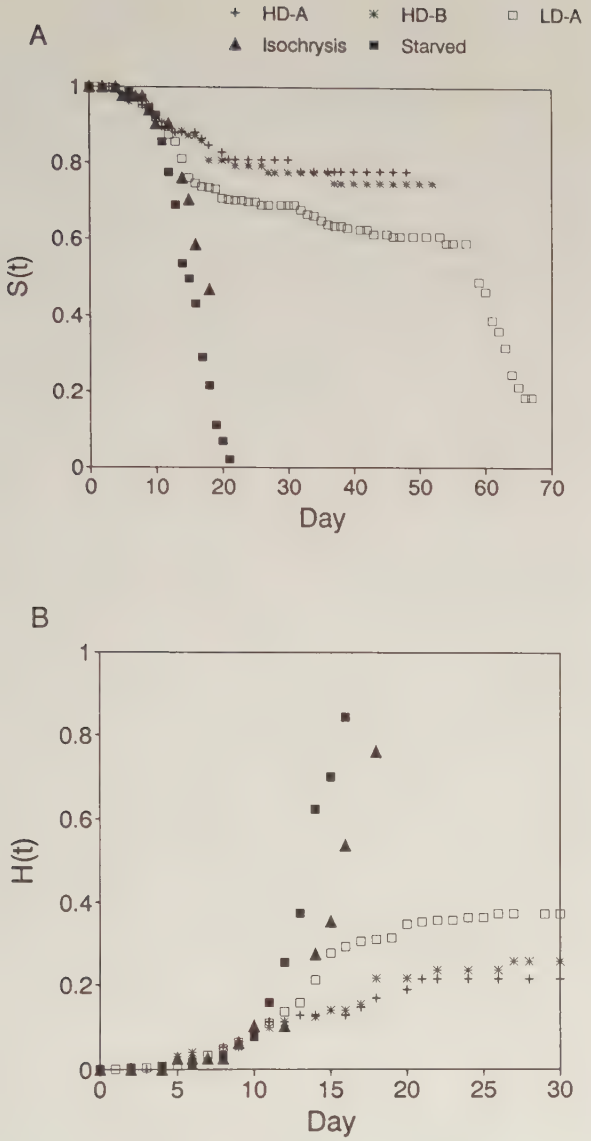

FIG. 7. (A) Survival function ($S_{(t)}$) of each larval group for the duration of the experiment and (B) the corresponding cumulative Hazard function ($H_{(t)}$) for the first 30-d of each treatment.

TABLE 3. Parameters estimated for the relationship between the Hazard function ($\ln(H_t)$) and time (ln(Day)) for the first two moults (days 5–25) in each experiment. The time axis (ln(Day)) was lagged by 5 d in order to avoid the indetermination in $\ln(H_t)$ when $H_t = 0$, i.e. no mortalities.

	n	Slope (β) (SE)	Intercept $-\ln(\lambda)$	R^2
HD-A	16	1.747 (0.179)	−6.798	0.871
HD-B	15	1.439 (0.084)	−5.886	0.956
LD-A	20	2.27 (0.116)	−7.812	0.955
Isochrysis-fed	11	2.93 (0.307)	−9.055	0.91
Starved	17	3.342 (0.261)	−9.508	0.916

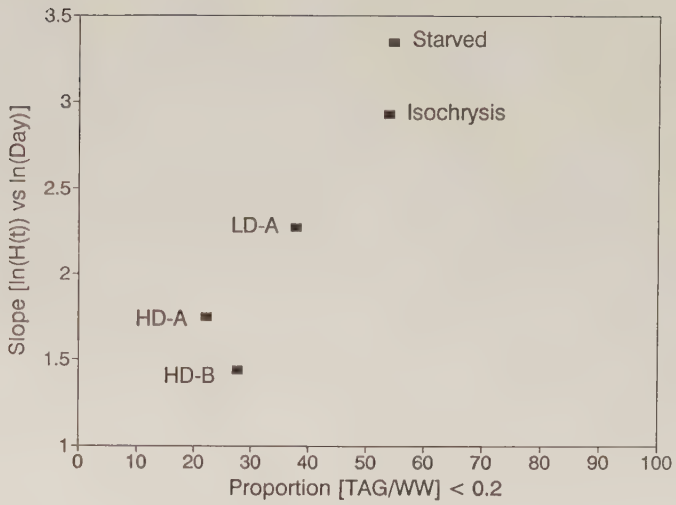

FIG. 8. Relationship between the risk of death (slope of $\ln(H_{(t)})$ vs. ln(Day)) and the relative proportions of larvae in poor condition in each feeding treatment.

premoult, and (d) the ecdysis periods (Anger and Dawirs 1981; Gore 1985). The minimal feeding time to reach the threshold in organic reserves and to initiate the premoult independently of further food supply is defined as the reserve saturation point (RSP) (Anger and Dawirs 1981). The same authors also defined the maximum time allowed for accumulation of reserves before the initiation of the premoult phase as the point of nonreturn (PNR). If the reserves threshold has not been reached at the PNR the larva may not survive ecdysis or may not be able to initiate the next postmoult phase, a phenomenon analogous to the "point of nonreturn" described for fish larvae (Blaxter 1969).

In all feeding conditions, the absolute amount of TAG per larvae was low, indicating the priority of development and growth over the accumulation of reserves early in development. However, our results present evidence of a pattern in the management of organic reserves similar to that described by Anger and Dawirs (1981) for *Hyas* spp. larvae. The larvae appeared depleted of TAG reserves at hatching, but within days (<5) the TAG increased to a maximum, indicative of immediate feeding after hatching. The absence of a well-defined cyclic pattern in the TAG/WW ratio in the HD-A larvae can be interpreted as evidence of the independence of moulting success on TAG reserves. However, under adequate feeding conditions the RSP can amount to only 30% of the intermoult period (Anger and Dawirs 1981), or only 3 d posthatch in the HD-A larvae (first moult after 10 d, 30% of 10 = 3 d). Thus, sampling every second day for biochemical analysis may have biased the data and prevented the detection of a clear pattern. Conversely, the variation can be explained by assuming that the food ration (and quality) in the HD-A experiment was adequate to permit minimal growth, development, and successful moulting, i.e. the HD-A larvae were less dependent on the internal storage of organic resources. This is supported by the observation of higher TAG/WW values and cyclic patterns during the first two moults in the slower growing/developing larvae of the HD-B and LD-A regimes. The absence of a clear pattern after the third cycles would result from asynchronous development among larvae, as the mixing of various development stages suggests.

The most significant result of the experiment is the relationship observed between the general condition of the larval groups

(i.e. proportion of larvae in poor TAG condition) and the risk of death (slope of the Hazard function) during the early moult periods. The simplest explanation is that insufficient organic energy, due to low TAG, results in larval death at moult. Other lipid classes (such as fatty acid or sterol esters and hydrocarbons) were rarely present or only in trace amount.

The prolonged intermoult (the extension of the RSP point) in the LD-A larvae also has the indirect effect of increasing the probability of observing larvae with relatively low TAG content and larvae that cannot secure the minimal energy required by the time of the PNR. This is also the case for the *Isochrysis*-fed and the Starved larvae. The effect is demonstrated by the frequency distributions of the individual condition indices (TAG/WW). The distributions are less variable in the HD-A and HD-B larvae than for larvae in LD-A, which also present a higher proportion of larvae in lower classes and must be recognized as a less favourable ration. The fact that we did not find significant differences in the frequency distributions of the groups (Kolmogorov–Smirnorv tests) does not invalidate the relationship. In fact the results of the tests are not surprising given the lower number of observations (n = 17, 18, and 24 for HD-A, HD-B, and LD-A, respectively), i.e. degrees of freedom for the test. A similiar approach, i.e. modification of the frequency distributions, was also proposed to evaluate changes in the physiological condition (i.e. lipid content) of individual larvae of the bivalve *Bankia gouldi* (Gallager and Mann 1986).

Overall, our results support the suggestion that the relative condition index among cohorts leads to useful estimates of survival probability and provides a possible method to compensate for the shortcomings of mortality estimates based strictly on larval abundance values (Taggart and Leggett 1987; Taggart and Frank 1990).

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Appendix 1

An empirical model for the intermoult TAG cycle was prepared using the Anger et al. (1989, fig. 4, p. 235) C/N ratio data for crab (*H. araneus*) larvae. The changes in C/N ratios over time were interpreted as indicative of lipid accumulation during the first part of the cycle (increasing C/N) with a shift to relative higher protein accumulation (declining C/N) at the premoult stages (Anger et al. 1989).

A simple data set was created by using the median of the daily values (scaled between 0 and 1) for zoea I larvae. The relationship was best described by an exponential model:

$$\mathrm{TAG} = \alpha[D - (1/D_{tg})]\exp[-\beta(D^2/D_{max})]$$

where TAG (in arbitrary units) is a function of the time in days (D) from the beginning of the experiment, the time at the maximum D_{tg}, i.e. at the reserves saturation point (30–40% of the intermoult; Anger and Dawirs 1981), and the duration of the stage D_{max}. The coefficients α and β were estimated by non-linear regression ($r^2 = 0.98$) analysis (NLIN; SAS 1988).

The expression was used to generate hypothetical TAG profiles for the shrimp larvae based on the differences in the moult cycles observed during the rearing experiments (Table 3; for the *Artemia*-fed larvae):

Diet	D_{tg} (d) increment = 0.3	D_{max} (d) increment = 1
HD-A	3–3.9	10–12
HD-B	3.9–4.8	10–13
LD-A	5.1–6	13–16

Variability at the beginning and asynchronous larval development were further represented by lagging D by 0.25 d up to 1 d ($D + 0.25$, $D + 0.5$,..., $D + 1$). Although the slightly slower growth of the HD-B larvae is reflected by the larger values of D_{tg}, variability in growth rates, and its possible influence in the TAG content, is not considered in the model so that the outputs represent only the simplest cases. The extremes in the simulated cycles corresponding to each experiment are provided (Fig. A.1) along with the measured daily mean TAG/WW values for the first moult cycle (from Fig. 4). Except for the high TAG/WW observed in LD-A, the measured TAG/WW ratios fit well in the limits of the simulated curves.

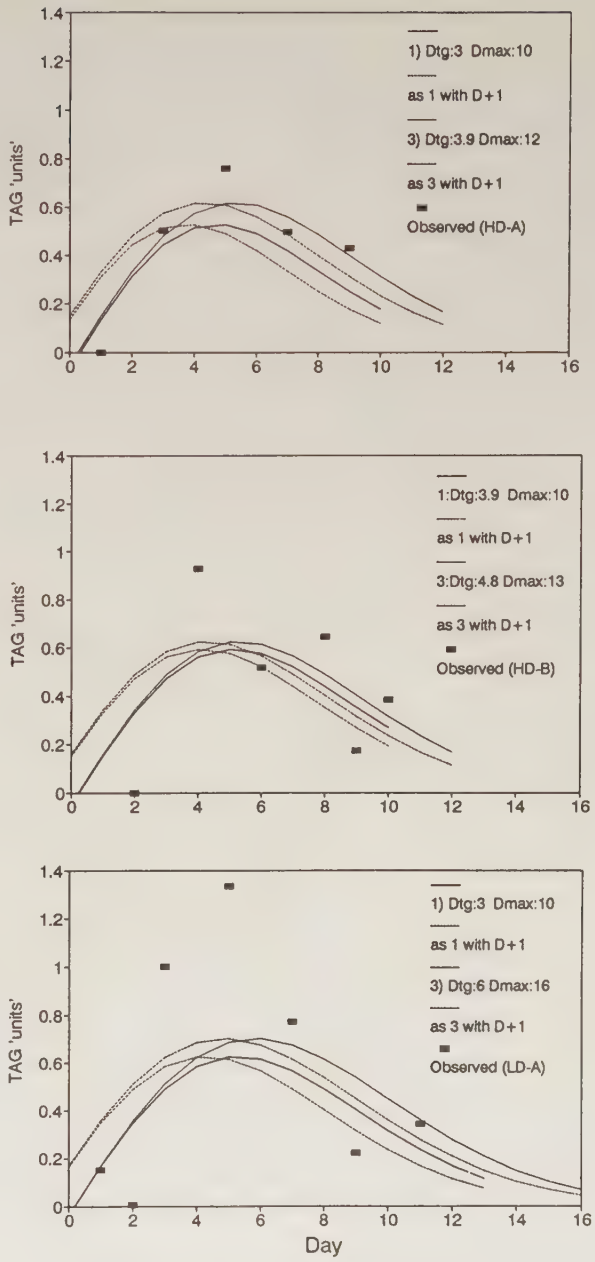

FIG. A.1. Simulated TAG cycles produced for each *Artemia* diet experiment with the corresponding daily mean TAG/WW ratios observed during the first moult cycle for each treatment.

We further sampled (every second "day") mixtures of simulated curves prepared for each experiment and constructed frequency distributions of the data for comparison with the frequency distributions derived from the observations (Fig. A.2; to be compared with Fig. 5, top panel). Although the simulated data do not reproduce the range of dispersion observed in the measured TAG/WW ratios, the patterns are consistent with bimodal distributions and an increase in the proportion of val-

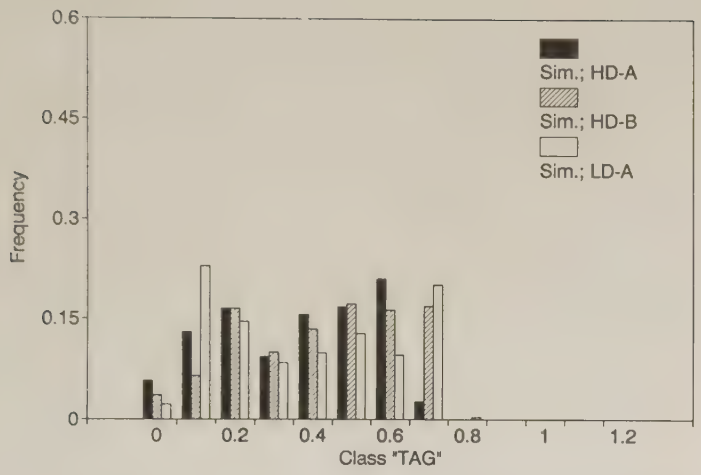

FIG. A.2. Frequency (relative) distribution generated by sampling mixtures of simulated cycles corresponding to each *Artemia*-fed larval group.

ues in the lower classes intervals from HD-A to LD-A. Furthermore, a Kolmogorov–Smirnorv test revealed that, except for HD-B, there were no significant differences between the simulated and observed frequency distributions ($P > 0.05$).

Phototrophic Picoplankton in Lakes Huron and Michigan: Abundance, Distribution, Composition, and Contribution to Biomass and Production

Gary L. Fahnenstiel and Hunter J. Carrick

Great Lakes Environmental Research Laboratory, National Oceanic and Atmospheric Administration, 2205 Commonwealth Blvd., Ann Arbor, MI 48105, USA

Fahnenstiel, G. L., and H. J. Carrick. 1992. Phototrophic picoplankton in Lakes Huron and Michigan: abundance, distribution, composition, and contribution to biomass and production. Can. J. Fish. Aquat. Sci. 49: 379–388.

The phototropic picoplankton communities of Lakes Huron and Michigan were studied from 1986 through 1988. Abundances in the surface-mixed layer ranged from 10 000 to 220 000 cells·mL^{-1} with a seasonal maximum during the period of thermal stratification. During thermal stratification, maximum abundances were generally found within the metalimnion/hypolimnion at depths corresponding to the 0.6–6.0% isolumes. The picoplankton community was dominated by single phycoerythrin-containing (PE) *Synechococcus* (59%) with lesser amounts of chlorophyll fluorescing cells (21%), PE colonial *Synechococcus*-like cells (11%), other PE colonial Chroococcales (6%), and other cells (3%). Single PE *Synechococcus* was abundant throughout the year whereas chlorophyll-fluorescing and colonial cyanobacteria were more abundant during the periods of spring isothermal mixing and summer stratification, respectively. Picoplankton accounted for an average of 10% (range 0.5–50%) of phototrophic biomass. Phototrophic organisms that passed 1-, 3-, and 10-μm screens were responsible for an average of 17% (range 6–43%), 40% (21–65%), and 70% (52–90%) of primary production. Maximum contributions of <1, <3, and <10 μm size fractions occurred during the period of thermal stratification. Primary production by phototrophic picoplankton was found to equal production in the <1 μm size fraction.

De 1986 à 1988, on a étudié les communautés de picoplancton phototrophe des lacs Huron et Michigan. Dans la couche de brassage de surface, l'abondance variait de 10 000 à 20 000 cellules·mL^{-1} et atteignait son maximum lors de la période de stratification thermique, en particulier dans le métalimnion et l'hypolimnion à des profondeurs correspondant aux isovolumes allant de 0,6 à 6,0 %. La communauté picoplanctonique était en grande partie composée de *Synechococcus* unicellulaires (59 %) contenant de la phycoérythrine (PE); elle comprenait aussi de moindres quantités de cellules fluorescentes (21 %), de cellules coloniales semblables à *Synechococcus* contenant de la PE, d'autres Chroococcales coloniales contenant de la PE (6 %) ainsi que d'autres types de cellules (3 %). Les populations de *Synechococcus* unicellulaires contenant de la PE étaient abondantes pendant toute l'année, tandis que les populations de cyanobactéries fluorescentes et coloniales étaient plus abondantes pendant les périodes de brassage printanier de l'isotherme et de la stratification estivale, respectivement. Le picoplancton représentait en moyenne 10 % (écart 0,5 à 50 %) de la biomasse planctonique. Les organismes phototrophes non retenus dans des cribles de 1, 3 et 10 μm étaient à l'origine, en moyenne, de 17 % (écart 6 à 43 %), 40 % (écart 21 à 65 %) et 70 % (écart 52 à 90 %) de la production primaire, respectivement. L'apport maximum des classes dimensionnelles de <1, <3 et <10 μm a été décelé lors de la période de stratification thermique. On a noté que la production primaire par le picoplancton phototrophe était égale à la production de la classe dimensionnelle de <1 μm.

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During the past decade, the importance of phototrophic picoplankton as significant contributors to primary production and phototrophic biomass in a wide variety of environments has been realized (see reviews by Stockner and Antia 1986; Stockner 1988). The contribution of picoplankton to primary production and phototrophic biomass increases in oligotrophic environments where it can be as much as 90% of total primary production (Li et al. 1983; Stockner and Antia 1986). Picoplankton communities are generally dominated by cyanobacteria of the genus *Synechococcus* and chlorophyll-fluorescing cells of a more uncertain taxonomic origin (Waterbury et al. 1979; Caron et al. 1985; Murphy and Haugen 1985; Glover et al. 1986; Chisholm et al. 1988). *Synechococcus* typically are abundant throughout most of the photic zone whereas chlorophyll-fluorescing cells become more abundant at the base (Murphy and Haugen 1985; Glover et al. 1986). Much of this picoplankton production appears to be consumed within the photic zone by small heterotrophic protozoans (Fahnenstiel et al. 1991a).

Most information regarding phototrophic picoplankton is from studies in the marine environment (Stockner and Antia 1986). In freshwater systems, previous studies have focused primarily on a specific component of the community (Caron et al. 1985; Fahnenstiel et al. 1986; Weisse 1988; Weisse and Munawar 1989; Fahnenstiel et al. 1991a, 1991b), on the contribution of picoplankton to biomass or production (Craig 1984;

Pick and Caron 1987; Stockner 1987), or the role of picoplankton in the food web (Stockner and Shortreed 1989). Only one study has described the abundance, distribution, and composition of the entire phototrophic picoplankton community (Nagata 1986).

The large, deep, oligotrophic nature of Lakes Michigan and Huron make them particularly good environments to study phototrophic picoplankton communities for comparisons with the marine environment. The microbial food web of Lake Michigan has received much attention in the past 5 yr with studies on the role of heterotrophic picoplankton (Scavia et al. 1986; Scavia and Laird 1987) as well as on the abundance, composition, and biomass of nanoplankton and microplankton communities (Carrick and Fahnenstiel 1989, 1990). Information on phototrophic picoplankton is needed to conceptualize carbon flow within the microbial food web of Lake Michigan. This study was initiated to describe the phototrophic picoplankton community of Lakes Huron and Michigan with a focus on abundance, composition, and contribution to primary production.

Materials and Methods

Sampling was conducted on 26 days at two offshore stations in Lake Huron (northern: 45°25′N, 82°55′W, maximum depth 150 m; southern: 43°54′N, 82°21′W, maximum depth 70 m) and on 15 days at a single station in Lake Michigan (43°1′N, 86°36′W, maximum depth 100 m) from March 1986 to November 1988. The biological, chemical, and physical conditions at these offshore stations are representative of general offshore conditions in both lakes (Lesht and Rockwell 1985; Makarewicz 1985, 1987; Laird et al. 1987). Total phosphorus concentrations range from 0.1 to 0.3 μM and soluble reactive phosphorus concentrations are at or below the detection level (<0.01 μM) during summer thermal stratification (Lesht and Rockwell 1985; Laird et al. 1987). Nitrate concentrations in both lakes generally exceed 15 μM throughout the year. Silica concentrations decrease from approximately 15–30 μM during the spring mixing period to ≤5–15 μM in Lake Michigan and 5–15 μM in Lake Huron during thermal stratification (Lesht and Rockwell 1985; Laird et al. 1987). Phytoplankton chlorophyll concentrations in the surface-mixed layer range from 0.5 to 3.0 $mg \cdot m^{-3}$ with highest values during the spring mixing period (Lesht and Rockwell 1985; Fahnenstiel et al. 1989).

Water samples were collected with 5- or 10-L PVC Niskin bottles. Water column temperature profiles were measured with an electronic bathythermograph, and underwater scalar irradiation was measured with a Licor LI-193SB sensor and LI-188B integrating meter. Chlorophyll concentrations were determined fluorometrically from 90% acetone extracted samples (Strickland and Parsons 1972).

For this study, picoplankton were defined by microscopic analysis as organisms <3 μm in all dimensions. This size is slightly larger than the 2-μm definition used by Sieburth et al. (1978) but similar to the size used by Stockner and Antia (1986). Because almost all phototrophic picoplankton in Lakes Huron and Michigan are significantly <2 μm, either definition could be used in this study with similar results.

Water samples for microscopic analysis were transferred into 250-mL amber bottles and preserved with either glutaraldehyde (1% final concentration) buffered with sodium cacodylate (0.1 M final concentration) for picoplankton or with 0.3% Lugol's acid iodine for phytoplankton. Picoplankton samples were then cooled (~5°C) until duplicate slides were prepared within 24 h (Waterbury et al. 1979) and then frozen (−20°C). These slides were counted within a few days to minimize the fading of autofluorescence. Picoplankton biomass and composition on each slide were estimated by enumerating a minimum of 500 units (<5% counting error assuming Poisson statistics, Lund et al. 1958) using a Leitz Laborlux microscope (magnification 1250×) equipped to distinguish the dominant bright autofluorescent emission of an individual cell. To examine abundance and composition of picoplankton within thermal regions of the water column (surface-mixed layer, metalimnion, and hypolimnion) on a given date, individual values (typically three to five) from specific depths were averaged within each layer.

The dominant fluorescence of a cell allowed us to determine the general taxonomic position of each cell (Tsuji et al. 1986). Specifically, chlorophyll-dominant cells emitted far-red light (>660 nm) when excited with blue light (450 nm) or green light (530–560 nm). Phycoerythrin-dominant cells were characterized by yellow emission following blue excitation and by bright red fluorescence (600–660 nm) when excited with green light. Phycocyanin-rich cells were detected by very faint or no emission in the red (600–660 nm) and far-red (>660 nm) when excited with blue light and by very bright red emission with green excitation. The length and breadth of 20 individuals of each taxon were measured twice during each major season from projections of photomicrographs. Cell volumes were calculated for each taxon assuming geometric configurations, and these estimates were subsequently converted to carbon using the conversion factor of 121 fg $C \cdot \mu m^{-3}$ (Watson et al. 1977).

Our procedure for preparing and counting picoplankton cells may underestimate total picoplankton abundance due to fading of very weak chlorophyll-fluorescing cells (Chisholm et al. 1988) or destruction of delicate cells (Murphy and Haugen 1985). To examine these possibilities, on several occasions we compared picoplankton counts using our standard approach with counts made immediately on live samples. We did not detect any difference for phycobilin-containing cells, but some differences were noted for chlorophyll-fluorescing cells, as counts from our standard procedure were on average 83% of counts from live samples (range 65–118%). Also, on two occasions we compared our routine counts of phototrophic picoplankton with counts from a flow cytometer and detected no significant differences (Fahnenstiel et al. 1991b). Thus, we consider our estimates of picoplankton abundance to be reasonably accurate.

Phytoplankton were prepared and enumerated using the technique of Dozier and Richerson (1975). Subsamples (5–25 mL) of the preserved phytoplankton samples were filtered onto 0.22-μm filters, placed on microscope slides, and subsequently cleared with glutaraldehyde under low heat. Cleared filters were then permanently mounted with Permount and a coverslip. The dimensions of each taxon encountered was measured and converted to biovolume by applying appropriate geometric shapes. These estimates were subsequently converted to carbon using the formula of Strathman (1966) with separate conversions for diatoms and nondiatoms.

Primary production was estimated using the ^{14}C technique (Vollenweider 1974) following modifications outlined in Fahnenstiel et al. (1989). Water collected from either the surface mixing layer or in the vicinity of the deep chlorophyll layer was immediately dispensed into duplicate shaded 2-L polycarbonate botles, inoculated with 2–220 μCi (1 μCi = 37 kBq) of ^{14}C as $NaHCO_3$, and incubated for a short time (1–2 h) at ambient

temperature in a shipboard photosynthesis-irradiance incubator. Following the incubation, subsamples from the high-activity 2-L bottles were postfractionated through 1- and 3-μm Nuclepore membranes and 10-μm Nitex screen; a portion of unfractionated water was retained to serve as an estimate of total primary production. Very low vacuum pressure was used for all of the fractionations (<50 mm Hg (1 mm Hg = 133.322 Pa)). Three subsamples from each fraction (<1, <3, and <10 μm) were filtered onto membranes (0.22-μm Millipore), decontaminated with 0.5 mL of 0.5 N HCl for 4–6 h, placed in scintillation vials with 12 mL of scintillation cocktail, and assayed with a Packard Tri-Carb spectrometer. Counting efficiencies were determined by external standards.

Results

Seasonal Temperatures and Light Extinction Coefficients

Surface-mixed layer temperatures were similar in Lakes Huron and Michigan. In 1986–88 the spring isothermal mixing period lasted from March to late May/early June in the southern regions of the lakes and into June in the northern regions. The period of thermal stratification began in May/June and lasted into December when fall turnover occurred. Maximum surface temperatures occurred in the late July/August period in the southern regions of the lakes and in August in the northern regions. At this time, the surface-mixed layer temperatures were 20–24°C and the depth of the surface-mixed layer was approximately 10–15 m. For the purposes of discussion, thermal stratification was divided into two periods: intermediate stratification when surface-mixed layer temperatures were from 4 to 15°C and midstratification when surface-mixed layer temperatures were >15°C (Fahnenstiel and Scavia 1987).

In both lakes, the light extinction coefficient (PAR) ranged from 0.14 to 0.23·m^{-1}; minimum values (0.14–0.17·m^{-1}) occurred in the July/early August period.

Picoplankton Abundance and Distribution

Although the study covered 3 yr, limited sampling in some years prevented us from examining annual variability. However, since major thermal periods were sampled several times during the study, representative conditions can be described (Table 1). In both lakes, picoplankton were abundant in the surface-mixed layer (10^3–10^5 cells·mL^{-1}) and demonstrated marked seasonal variability (Fig. 1 and 2; Table 1). Mean surface picoplankton abundance was 5.96×10^4 cells·mL^{-1} in Lake Michigan and 4.27×10^4 cells·mL^{-1} in Lake Huron whereas maximum surface abundance was 2.26×10^5 cells·mL^{-1} in Lake Michigan and 1.21×10^5 cells·mL^{-1} in Lake Huron. Generally, surface picoplankton abundances were low during isothermal mixing periods or during weak inversely stratified periods and then increased during thermal stratification (Fig. 1 and 2; Table 1). Abundances were similar in the northern and southern Lake Huron stations (Fig. 1), suggesting that horizontal variation in the offshore regions of the lake was minimal.

During summer thermal stratification, distinct vertical variation in picoplankton abundance was found with maximum abundance generally located at approximately 20–30 m within the metalimnion or upper hypolimnion (Fig. 3 and 4). With light extinction coefficients ranging from 0.14 to 0.17·m^{-1}, the depths of these maxima received 0.6–6.0% of surface irradiance. Maximum picoplankton abundances in both lakes (2.27×10^5 cells·mL^{-1} in Lake Michigan and 2.15×10^5 cells·mL^{-1} in Lake Huron) were found within the metalimnion. With one exception (Lake Huron, 5 August 1986), all vertical profiles of picoplankton abundance exhibited a subsurface maximum severalfold higher than the abundance of surface populations (deep to surface ratio 2.0–11.0) with the most pronounced maxima occurring in July/August.

Community Structure

We divided the picoplankton community into five groups based on pigment fluorescence, morphology, size of cell, and mode of growth (Table 2). The structure of the picoplankton communities was similar in both lakes (Fig. 1 and 2; Table 2) and was dominated by phycoerythrin-rich cyanobacteria of approximately 0.9 to 1.6 μm, which constituted approximately 80% of the total picoplankton community.

The most abundant cyanobacteria were single coccoid phycoerythrin-rich cyanobacteria of approximately 1.0 μm (Table 3), which constituted, on average, 50–60% of total abundance in both lakes (Fig. 1 and 2; Table 2). The maximum abundance of these cells was 1.89×10^5 cells·mL^{-1} in Lake Michigan and 1.78×10^5 cells·mL^{-1} in Lake Huron, with both

TABLE 1. Mean abundance of phototrophic picoplankton from major thermal periods and layers at two stations in Lake Huron and at a single station in Lake Michigan (n = number of sampling dates).

Lake	Thermal period	Layer	Mean abundance[a]	n
Michigan	Isothermal	Surface	26 000	7
	Stratification	Surface	70 900	8
	Stratification	Metalimnion	105 200	7
	Stratification	Hypolimnion	12 200	7
Mean			59 600	15
Huron	Isothermal	Surface	38 400	8
	Stratification	Surface	53 500	18
	Stratification	Metalimnion	65 400	15
	Stratification	Hypolimnion	15 500	10
Mean			42 700	26
Grand mean both lakes			51 150	41

[a]Units are cells·mL^{-1}.

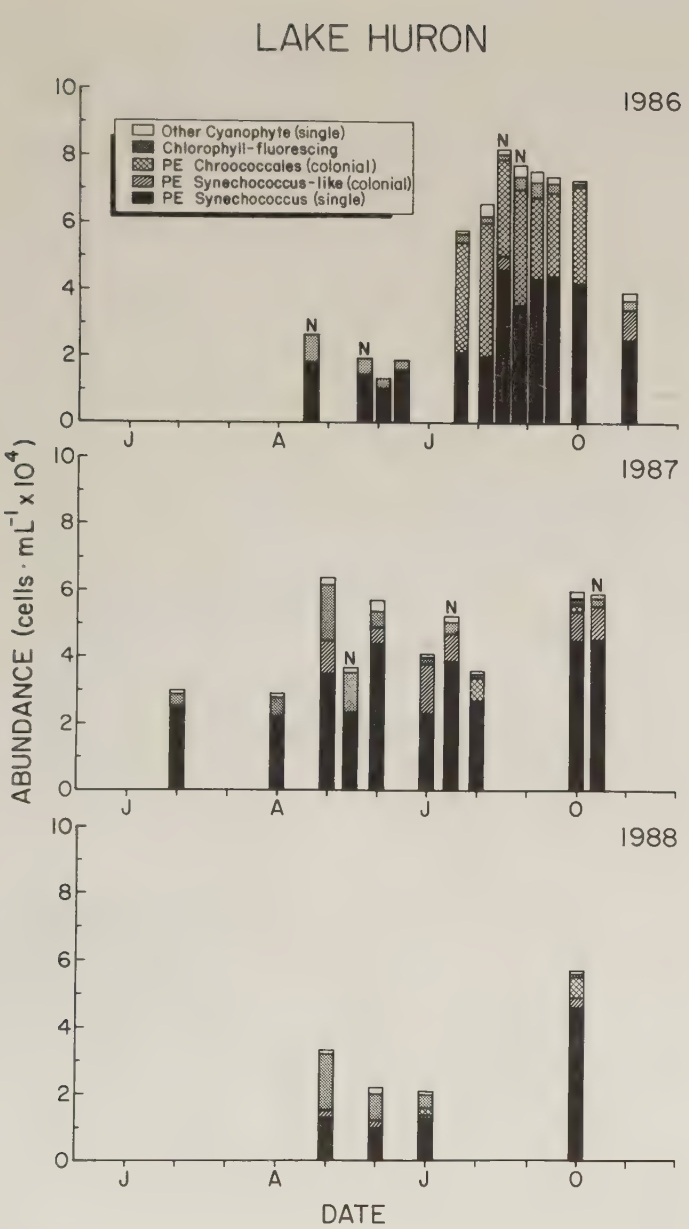

FIG. 1. Seasonal abundance and composition of phototrophic picoplankton from the surface-mixed layer at two stations in Lake Huron. N indicates data from northern Lake Huron station. The five groups of picoplankton are phycoerythrin-containing (PE) single *Synechococcus*, PE colonial *Synechococcus*-like cells, other PE colonial Chroococcales cells, chlorophyll-fluorescing cells, and other cyanophytes.

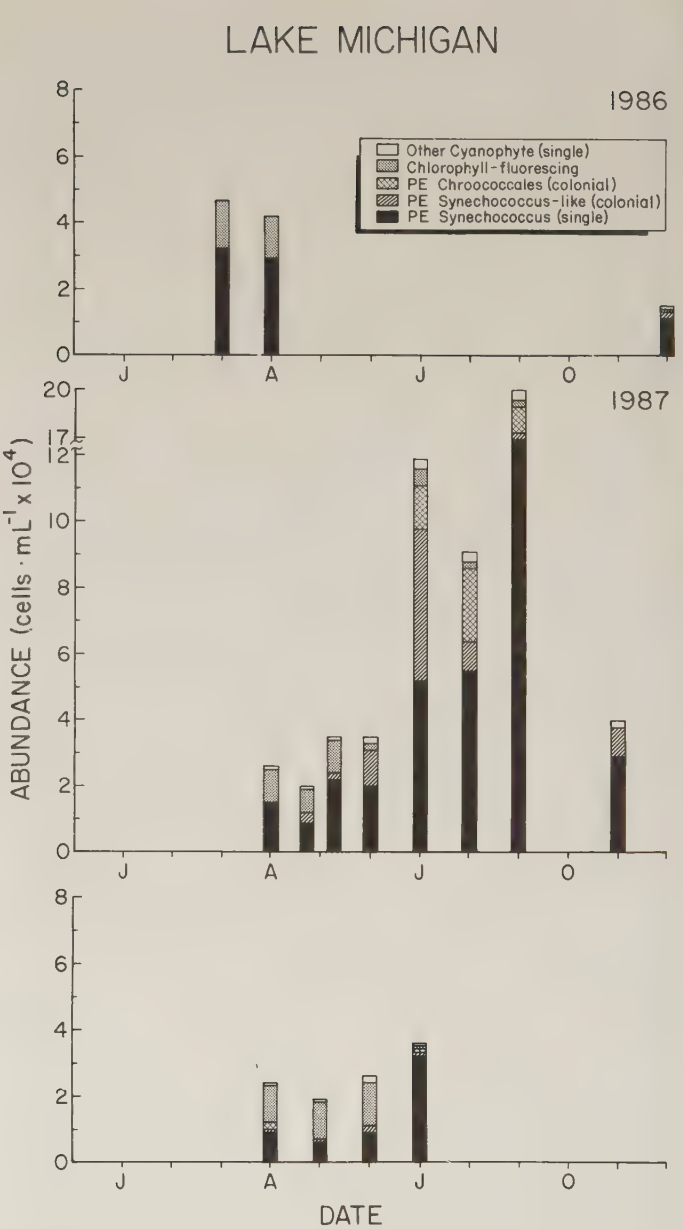

FIG. 2. Seasonal abundance and composition of phototrophic picoplankton from the surface-mixed layer at a single station in Lake Michigan. Picoplankton groups are as defined in Fig. 1.

maxima occurring within the metalimnion during thermal stratification. In culture, an isolate of these cells divided in one plane; therefore, we tentatively assigned these cells to the genus *Synechococcus* (Stanier et al. 1971). *Synechococcus* dominance was not confined spatially or temporally in either lake, but rather dominated at all stations throughout the study (Table 2).

Although picoplankton communities were dominated generally by single-celled *Synechococcus*, other phycoerythrin-containing cyanobacteria became abundant at specific times of the year (Fig. 1 and 2; Table 2). During thermal stratification, colonial or aggregate cyanobacteria become abundant, and in some cases, even dominant. The percent contribution of colonial cyanobacteria to total picoplankton abundance increased dramatically from approximately 5% during the spring isothermal period to over 20% during thermal stratification. Most of the large increase in total picoplankton abundance during thermal stratification can be attributed to this increase in colonial forms (Fig. 1 and 2), particularly in Lake Huron. On average, colonial phycoerythrin-containing cyanobacteria contributed 21.1 and 13.6% of total picoplankton abundance in Lakes Michigan and Huron, respectively.

These colonial cyanobacteria were divided into two groups based on cell morphology. The first and most abundant group of colonial cyanobacteria (Table 2) was morphologically similar to the single *Synechococcus* group described above, differing only in the colonial mode of growth. This coccoid colonial *Synchococcus*-like group was separated because we were unable to produce any colonial aggregations in our culture of the single *Synechococcus* isolate under a variety of conditions, and the ecology of these two groups is probably different. The

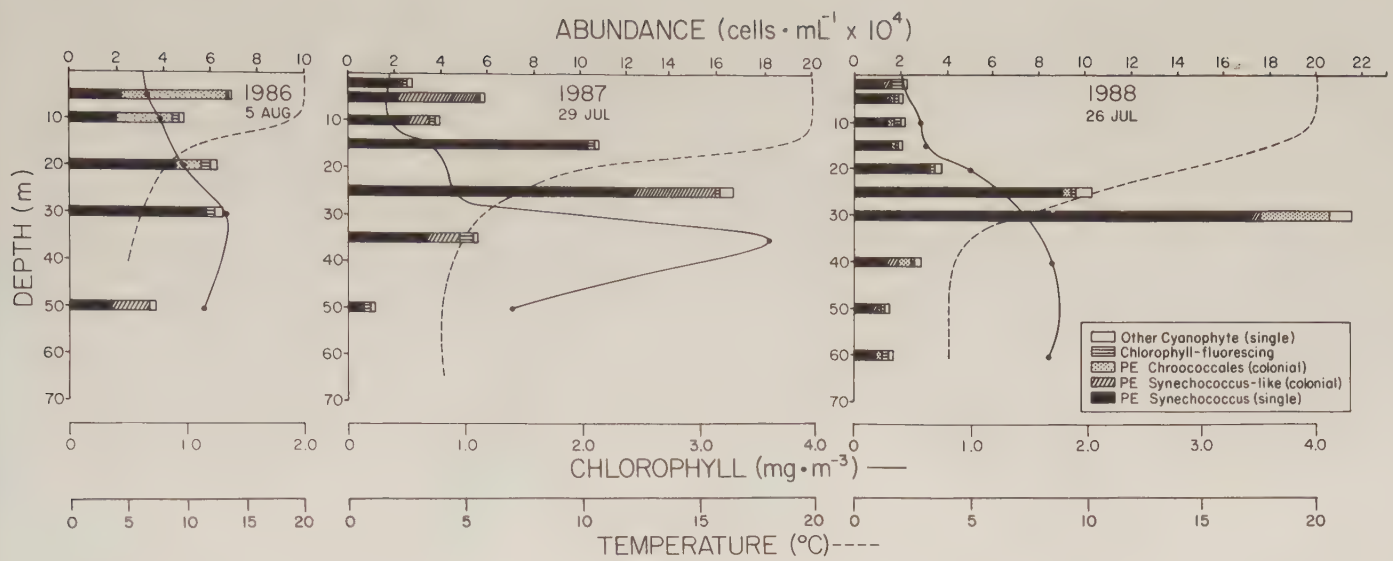

FIG. 3. Vertical distribution and composition of phototrophic picoplankton during thermal stratification at a station in southern Lake Huron (43°54′N, 82°21′W) on 5 August 1986, 29 July 1987, and 26 July 1988. The solid line represents chlorophyll concentration and the broken line represents temperature. Picoplankton groups are as defined in Fig. 1.

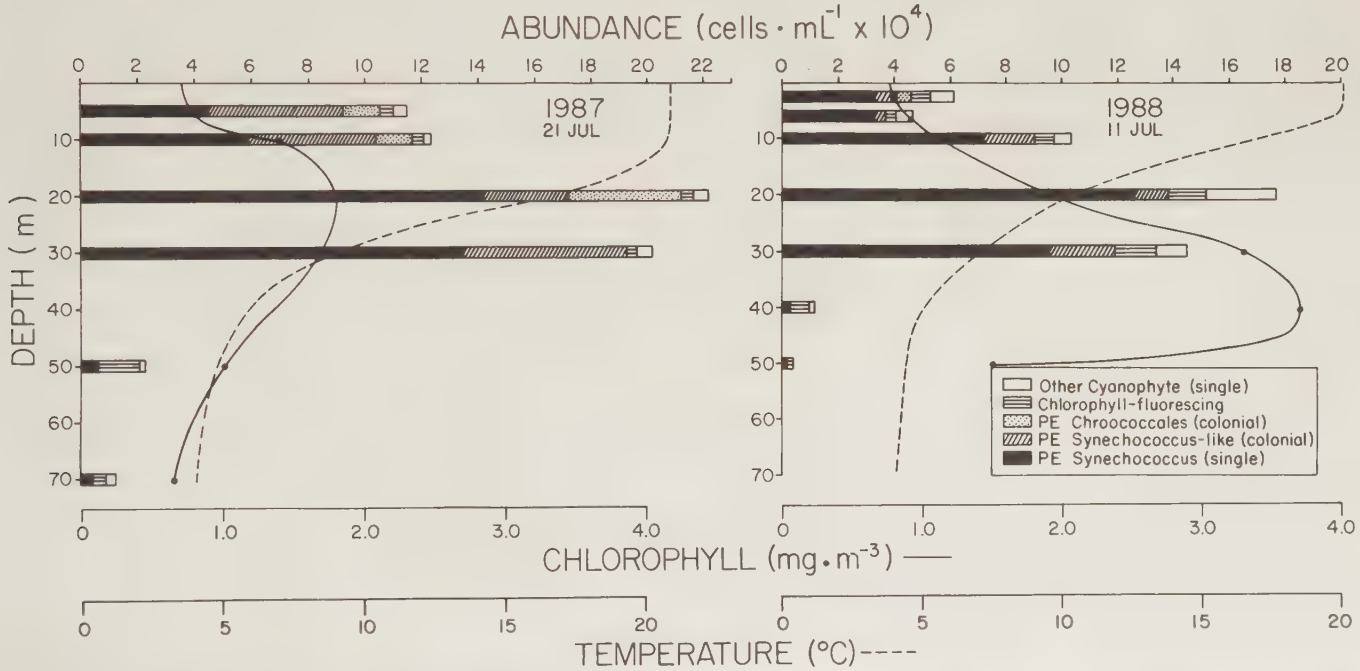

FIG. 4. Vertical distribution and composition of phototrophic picoplankton during thermal stratification at a station in southern Lake Michigan (43°1′N, 86°36′W) on 21 July 1987 and 11 July 1988. The solid line represents the chlorophyll concentration and the broken line represents temperature. Picoplankton groups are as defined in Fig. 1.

maximum abundance of this colonial type of cyanobacteria was 5.80×10^4 cells·mL^{-1} in Lake Michigan and 4.48×10^4 cells·mL^{-1} in Lake Huron. These cells contributed on average 15 and 7.7% of total picoplankton abundance in Lakes Michigan and Huron, respectively.

The second type of colonial cyanobacteria was a more diverse group of taxa which belonged to the order Chroococcales, but were distinct from the *Synechococcus*-like colonial form described above. The vast majority of these cells was similar to the old taxonomic genera of *Aphanothece* and *Microcystis* (Prescott 1964). Rod-shaped colonial cells were particularly abundant in Lake Huron in 1986 (Fig. 1). As with the *Synechococcus*-like colonies, these colonial forms were most abundant during thermal stratification (Fig. 1 and 2). On average, only 5.9 and 6.1% of total picoplankton abundance in Lakes Huron and Michigan, respectively, was contributed by this group of phycoerythrin-containing Chroococcales cyanobacteria (Table 2).

A group of red-fluorescing cells contributed substantially to total picoplankton abundance. This red fluorescence was emitted at wavelengths >660 nm, and therefore, we assume that this fluorescence was due to chlorophyll *a* or a chlorophyll

TABLE 2. Percent composition of major picoplankton groups during the isothermally mixed and thermally stratified periods in Lakes Huron and Michigan. The regions of the water column are defined as surface-mixed layer (S), metalimnion (M), and hypolimnion (H).

Lake	Thermal period	Region	PE single *Syn.*[a]	PE colonial *Syn.*-like[b]	PE colonial *Chrooc.*[c]	Chl-fluor.[d]	Other[e]
Michigan	Isothermal	S	54.8	5.2	0.9	35.9	2.2
	Stratification	S	61.0	15.5	6.2	13.3	4.0
	Stratification	M	53.7	18.1	9.0	15.3	3.9
	Stratification	H	43.5	14.7	4.7	36.1	1.1
Mean			53.8	15.0	6.1	24.4	2.8
Huron	Isothermal	S	57.9	5.4	0.0	34.2	2.5
	Stratification	S	63.5	10.4	13.6	9.3	3.2
	Stratification	M	74.3	6.6	5.4	9.9	3.8
	Stratification	H	59.1	8.2	3.9	25.1	4.8
Mean			65.1	7.7	5.9	17.5	3.8
Grand mean both lakes			59.5	11.4	6.0	21.0	3.1

[a]Phycoerythrin-containing single *Synechococcus*.
[b]PE colonial *Synechococcus*-like cells.
[c]Other PE colonial Chroococcales.
[d]Chlorophyll-fluorescing cells.
[e]Other cyanophytes.

TABLE 3. Mean dimensions and biovolume of dominant picoplankton.

Dominant piciplankton[a]	Mean biovolume (μm^3)	Mean dimensions (μm)
PE single *Synechococcus*	0.56	Diameter 1.0
PE colonial *Synechococcus*-like	0.70	Diameter 1.1
PE colonial Chroococcales	0.71	Length 1.6, width 0.9
Chlorophyll-fluorescing	0.96	Diameter 1.2

[a]*Picoplankton groups are as defined in Table 2.*

TABLE 4. Mean and range of picoplankton carbon expressed as percentage of total phototrophic carbon during the periods of isothermal mixing and thermal stratification.

Lake	Thermal period	Depth	Mean percent and range
Michigan	Isothermal	Surface	4.4 (1.4–12.7)
	Stratification	Surface	12.1 (2.8–30.0)
	Stratification	Metalimnion	20.0 (1.3–49.6)
	Stratification	Hypolimnion	4.6 (0.5–13.5)
Mean			11.5 (0.5–49.6)
Huron	Isothermal	Surface	11.1 (4.4–23.9)
	Stratification	Surface	11.0 (1.3–31.5)
	Stratification	Metalimnion	11.1 (3.7–23.4)
	Stratification	Hypolimnion	2.6 (1.4–4.8)
Mean			10.4 (1.3–31.5)
Grand mean both lakes			10.2

a-like pigment (Chisholm et al. 1988). These small (1.2 μm) single chlorophyll-fluorescing cells constituted 24.4 and 17.5% of total picoplankton abundance in Lakes Michigan and Huron, respectively. Maximum abundance of these cells occurred during the isothermal mixing periods, with abundances of 1.68×10^4 cells·mL^{-1} in Lake Michigan and 1.93×10^4 cells·mL^{-1} in Lake Huron (Table 2). These cells were also relatively abundant in the hypolimnion during thermal stratification (Table 2); however, there was no pronounced abundance peak within the hypolimnion (Fig. 3 and 4).

Unclassified or other types of picoplankton constituted only a small percent of total picoplankton abundance (Fig. 1–4; Table 2). These cells were mostly single rod-shaped phycoerythrin-containing cyanobacteria or phycocyanin-containing cyanobacteria.

Size and Biomass Estimates

As noted above, the picoplankton community was dominated by very small individuals ranging in mean size (longest dimension) from 1.0 to 1.6 μm (Table 3). We did not detect any consistent change in picoplankton size between lakes or across seasons. The mean and range of picoplankton carbon were similar in Lakes Huron and Michigan: mean 4.3 mg C·m^{-3} (0.8–16.8) in Lake Huron; mean 5.7 mg C·m^{-3} (0.4–18.0) in Lake Michigan. Despite their small size, picoplankton contributed significantly to total pelagic phototrophic carbon, with an overall average of 10.2% (Table 4). The contribution of picoplankton carbon to phototrophic biomass was variable, ranging from a low of 1% during mixing periods to maximum values of over 30% during midstratification (Table 4). More seasonal variability in percent picoplankton carbon was apparent in Lake Michigan than in Lake Huron (Table 4). The maximum contribution of picoplankton to total phototrophic carbon was 50%, which was observed in Lake Michigan in August 1987.

Fractionated Primary Production

Phytoplankton primary production in both lakes was dominated by organisms passing a 10-μm screen (Fig. 5 and 6). For surface-mixing layer communities, the overall contributions to carbon fixation among the <1, <3, and <10-μm size fractions were 17, 40, and 70%, respectively (Table 5). The per-

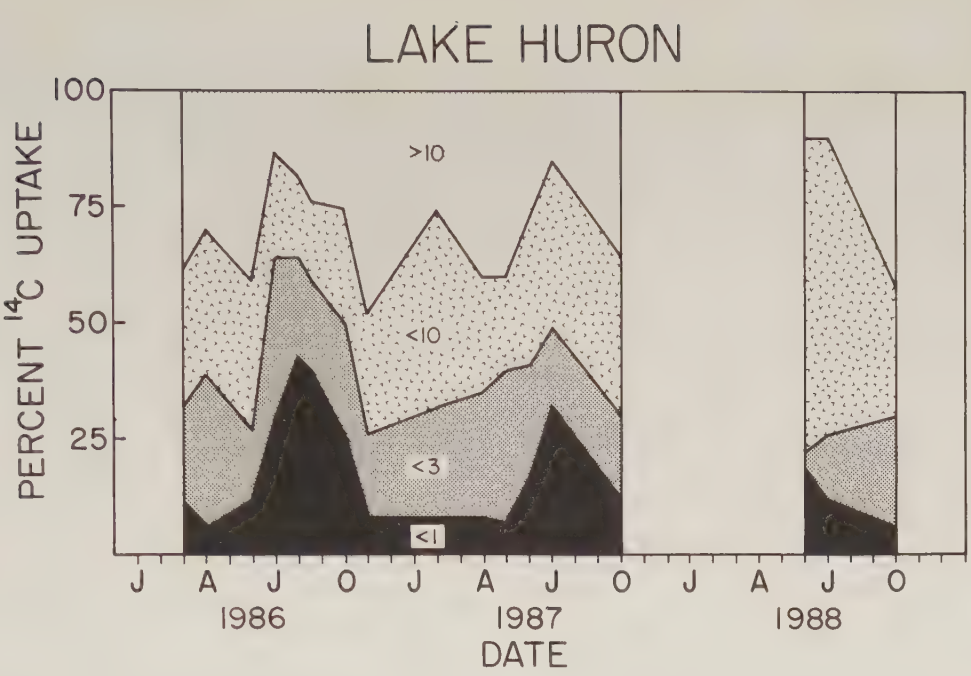

FIG. 5. Percent of primary production by the <1, <3, and <10 μm size classes for surface-mixed layer communities from a station in southern Lake Huron (43°54′N, 82°21′W).

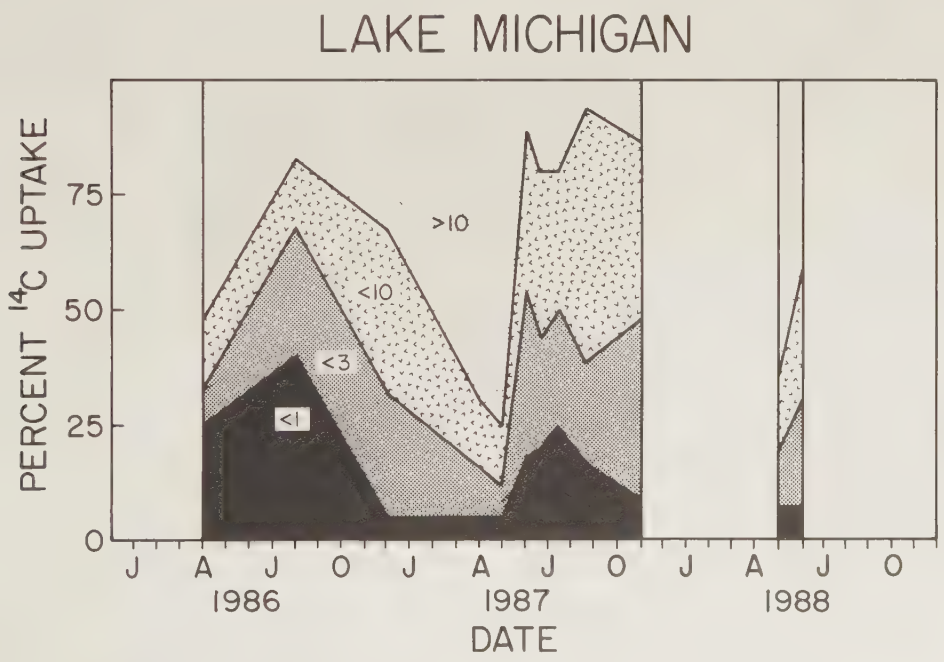

FIG. 6. Percent of primary production by the <1, <3, and <10 μm size classes for surface-mixed layer communities from a station in southern Lake Michigan (43°1′N, 86°36′W).

cent of carbon fixation by each fraction showed a consistent seasonal pattern in each lake, with the greatest contribution of each fraction occurring during thermal stratification (Fig. 5 and 6). During the midstratification period, approximately 30, 50, and 80% of primary production was attributable to organisms passing .1-, 3-, and 10-μm screens, respectively (Table 5). Within the deep chlorophyll layer, the contributions of the <1 and <3 μm size fractions were similar to the surface communities.

Discussion

The phototrophic picoplankton communities of Lakes Michigan and Huron exhibit striking similarities to picoplankton communities in other freshwater and marine environments, but also exhibit notable differences. In many respects, especially composition and distribution, the picoplankton communities in these lakes are a cross between communities previously described from freshwater and oceanic environments, which is

TABLE 5. Mean percent carbon fixation by the <1, <3, and <10 μm size classes from the surface-mixed layer in Lakes Huron and Michigan (n = number of sampling dates).

Lake	Thermal period	<1 μm	<3 μm	<10 μm	n
Michigan	Isothermal	9.2	22.6	41.1	5
	Internal stratification	12.9	43.3	78.9	4
	Midstratification	25.8	52.3	86.6	4
Mean		15.4	38.1	67.5	13
Huron	Isothermal	7.9	36.0	61.9	7
	Internal stratification	13.2	35.5	701.	8
	Midstratification	32.1	51.3	82.1	7
Mean		17.5	40.8	71.6	22
Grand mean both lakes		16.7	39.8	70.1	35

probably related to the deep oligotrophic nature of Lakes Huron and Michigan.

Like many other freshwater and marine picoplankton communities (Glover et al. 1985; Murphy and Haugen 1985; Fahnenstiel et al. 1986; Iturriaga and Mitchell 1986; Jochem 1988; Weisse 1988), single phycoerythrin-containing chroococcoid cyanobacteria of the genus *Synechococcus* dominated throughout the year. Maximum abundances of these cells were generally found within the metalimnion/upper hypolimnion during thermal stratification. These cells were morphologically similar to *Synechococcus* cells from Lake Superior (Fahnenstiel et al. 1986, G. Fahnenstiel, unpubl. data) and the type I group described by Johnson and Sieburth (1979) and Leppard et al. (1987). While single phycoerythrin-containing *Synechococcus* account for 60% of total phototrophic picoplankton in Lakes Huron and Michigan, other types of phototrophic picoplankton, particularly chlorophyll-fluorescing and colonial cyanobacteria, became abundant at certain times of the year. Previously described freshwater and marine communities did not exhibit this type of diversity.

Recent descriptions of freshwater picoplankton communities have failed to note abundant chlorophyll-fluorescing (red) cells of picoplankton size (0.5–2.0 μm). The phototrophic picoplankton community in Lake Constance was almost exclusively chroococcoid cyanobacteria (Weisse 1988), and phycoerythrin-containing cyanobacteria also dominated in Lakes Ontario and Biwa (Caron et al. 1985; Nagata 1986). During the summer in Lake Biwa, chlorophyll-fluorescing phytoflagellates of approximately 3–4 μm were noted, but these cells would not be included as picoplankton in most previous investigations. However, in Lakes Huron and Michigan, chlorophyll-fluorescing nonflagellated cells of approximately 1 μm become very abundant, and in some cases dominant, during isothermal mixing periods (Table 2; Fig. 1 and 2). Chlorophyll-fluorescing cells in this size range are abundant in many marine environments (Glover et al. 1985; Murphy and Haugen 1985; Chisholm et al. 1988; Jochem 1988; Li and Wood 1988). A shift in the picoplankton communities from cyanobacteria to chlorophyll-fluorescing reported for the Kiel bight of the western Baltic Sea (Jochem 1988) is similar to the one reported here. Chlorophyll-fluorescing cells are probably a more important constituent of freshwater picoplankton communities than has been previously reported.

Although red-fluorescing phototrophic picoplankton are abundant in the Great Lakes and in marine environments, these communities may be taxonomically very different. In the Great Lakes, the red-fluorescing community is dominated by *Chlorella*-like eukaryotes, and prochlorophytes do not appear to be present (Fahnenstiel et al. 1991b). However, in the marine environment, prochlorophytes are extremely abundant, particularly in oceanic regions, and the community of red-fluorescing cells probably is composed of both eukaryotes and prochlorophytes (Johnson and Sieburth 1982; Chisholm et al. 1988).

The importance of colonial picoplankton also may differ between freshwater and marine environments. Previous descriptions of marine picoplankton communities have not included significant abundances of colonial cyanobacteria. Single cyanobacteria dominate the surface waters of most marine communities (Johnson and Sieburth 1979; Glover et al. 1985; Murphy and Haugen 1985; Iturriaga and Mitchel 1986). The role of colonial picoplankton is unclear; they may not have been counted as picoplankton. For example, Li et al. (1983) noted aggregates of cyanobacteria of up to 96 cells in the tropical ocean, but did not mention their overall contribution to picoplankton abundance.

Whether aggregate or colonial cells of 1–2 μm size are included as picoplankton is related to one's definition of picoplankton. Picoplankton can be defined either by the size of the organism (Sicko-Goad and Stoermer 1984; Stockner and Antia 1986) or by the ability to pass through a certain size screen (Smith et al. 1985; Joint 1986). Because of the variability and unpredictability in filter fractionation material (Runge and Ohman 1982; Stockner et al. 1990), we have chosen the former definition to describe our picoplankton communities and we also have differentiated between single and colonial forms.

Colonial or aggregate cyanobacteria commonly occur in freshwater environments (Wetzel 1983; Reynolds 1984). In Lakes Huron and Michigan, colonial cyanobacteria, some morphologically similar to the single *Synechococcus*, were very abundant during summer stratification; in certain cases, the abundance of colonial forms exceeded that of single *Synechococcus* (Fig. 1 and 2). The majority of colonial cells occurred in small aggregates of 9–16 cells, and their relationship to the single cells is unclear. Environmental conditions in the summer, e.g. increased grazing and nutrient deficiency, may induce colonial growth or simply select for colonial species. Since colonial cyanobacteria of picoplankton size are common during summer stratification and have been counted as picoplankton in the past (Caron et al. 1985), the relationship between single and colonial forms merits further work.

The vertial distribution of picoplankton during thermal stratification in Lakes Huron and Michigan is similar to that reported for picoplankton from the marine environment. Maximum abundance of picoplankton generally occurred within the lower metalimnion/upper hypolimnion at depths of 20–30 m which corresponds to the 0.6–6.0% isolumes. In many marine systems, the maximum abundance of picoplankton during stratification occurred near the depths of similar isolumes (Glover et al. 1985; Murphy and Haugen 1985; Waterbury et al. 1986) although there is some variability (Glover et al. 1988). However, the vertical distribution of picoplankton in Lakes Huron and Michigan differs from that reported for freshwater environments where the maximum abundances typically occurred within the epilimnion (Nagata 1986; Pick and Caron 1987; Weisse 1988). This difference in freshwater communities is likely related to the relationship between light penetration and mixing depth (Waterbury et al. 1986); there is greater light penetration into the metalimnion/hypolimnion in Lakes Huron and Michigan than in the other lakes studied. However, differential

nutrient availability with depth also may influence the vertical distribution of picoplankton (Shortreed and Stockner 1990).

One striking difference in the vertical distribution of picoplankton in Lakes Huron and Michigan as compared with oceanic environments is the absence of a deep peak of chlorophyll-fluorescing cells. In oceanic environments, chlorophyll-fluorescing cells become very abundant and sometimes are dominant below the 1% isolume (Glover et al. 1985; Murphy and Haugen 1985; Chisholm et al. 1988). Although red-fluorescing cells may be taxonomically different between the Great Lakes and the oceanic regions, the absence of a deep peak in the Great Lakes is probably related to spectral light penetration. In oceanic environments, more blue light is available at depth than in freshwater environments, and chlorophyll-fluorescing cells are better adapted for growth in blue light (Glover et al. 1986).

The density and seasonal pattern of occurrence of picoplankton in Lakes Huron and Michigan resembles that found in many temperate freshwater and marine environments. The range of picoplankton abundance in surface waters (10 000 to 220 000 cells·mL^{-1}) of Lakes Huron and Michigan is similar to values reported for freshwater and shelf and slope marine environments. (Caron et al. 1985; Joint 1986; Nagata 1986; Waterbury et al. 1986; Jochem 1988; Weisse 1988) but is on the high end of abundances reported from oceanic environments (Davis et al. 1985; Murphy and Haugen 1985). In temperate aquatic environments, maximum seasonal picoplankton abundance occurs during thermal stratification (Krempin and Sullivan 1981; Caron et al. 1985; Joint 1986; Jochem 1988). The same pattern was observed in Lakes Huron and Michigan, where maximum abundance was found during the July/October period. If, however, colonial forms were not included as picoplankton, much of this pronounced seasonality would disappear, particularly in Lake Huron. It is unclear if the pronounced seasonal peak noted by other investigators was due to colonial or single forms.

Despite their small size, picoplankton are important contributors to phototrophic biomass and primary production in Lakes Huron and Michigan. On average, picoplankton accounted for 10% of total phototrophic biomass, and on some occasions during the summer as much as 50%. This contribution to phototrophic biomass is higher than values reported from a Chesapeake Bay subestuary (Ray et al. 1989), Lake Constance (Weisse 1988), and southern California coastal waters (Krempin and Sullivan 1981), but is in the range of values for Lake Ontario (Pick and Caron 1987), the Kiel Bight (Jochem 1988), the Baltic Sea (Larsson and Hagstrom 1982), and Lake Biwa (Nagata 1986).

Our size-fractionated ^{14}C uptake experiments demonstrated the importance of phototrophic organisms that pass through 1- and 3-μm screens (Fig. 5 and 6). However, because not all phototrophic organisms are precisely fractionated (Runge and Ohman 1982; Waterbury et al. 1986) and because larger phototrophs may be damaged and broken during filtration procedures (Bloem and Gilissen 1985; Goldman and Dennett 1985), the relationship between production in a specific size fraction and picoplankton production is not always clear.

To evaluate the use of size fractionation experiments for estimating picoplankton production, we compared direct estimates of phototrophic picoplankton production determined with track autoradiography and microscopic counts on several occasions with production in the <1 and <3 μm size fractions (G. Fahnenstiel, unpubl. data). On average, production by phototrophic picoplankton was similar to production in the <1 μm size fraction. Based on these comparisons and the results of this study, phototrophic picoplankton contribute an average of 17% of primary production in Lakes Huron and Michigan, but their contribution likely exceeds 40% during the period of thermal stratification.

In summary, phototrophic picoplankton are very abundant and significant contributors to phototrophic biomass and primary production in Lakes Huron and Michigan. Because of their importance, particularly during summer stratification, many of our ideas and models of food web structure and carbon flow in the Great Lakes need to be reevaluated. Budgets and analyses of community chlorophyll and carbon that include typical phytoplankton rate processes such as sedimentation, grazing, and growth must recognize the difference between larger, more traditional phytoplankton and picoplankton; otherwise, predictions on the control of phytoplankton community structure may be in error (Lehman 1988; Scavia et al. 1988) Furthermore, our results, combined with the results of Scavia et al. (1986) and Scavia and Laird (1987) on the importance of heterotrophic picoplankton, suggest that heterotrophic and phototrophic picoplankton are responsible for the majority of planktonic community metabolism during summer stratification in the upper Great Lakes. A similar central role of heterotrophic and phototrophic picoplankton has been suggested for the oceans (Pomeroy 1980; Fenchel 1988).

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Size Variation of *Neocalanus plumchrus* and *Neocalanus flemingeri* in a 20-yr Sample Series from the Gulf of Alaska

Charles B. Miller

College of Oceanography, Oregon State University, Corvallis, OR 97331, USA

John Fulton

Department of Fisheries and Oceans, Resource Services Branch, Pacific Biological Station, Nanaimo, B.C. V9R 5K6, Canada

and Bruce W. Frost

School of Oceanography, University of Washington, WB-10, Seattle, WA 98195, USA

Miller, C. B., J. Fulton, and B. W. Frost. 1992. Size variation of *Neocalanus plumchrus* and *Neocalanus flemingeri* in a 20-yr sample series from the Gulf of Alaska. Can. J. Fish. Aquat. Sci. 49: 389–399.

Prosome length of the resting stages of *Neocalanus plumchrus* and *Neocalanus flemingeri* varied significantly, but modestly, among years at Station P in the Gulf of Alaska. The range of variation over 20 yr was small relative to the known geographic variation. Among-year length variations were significantly greater than within-year variations, implying similarity of year-to-year variation in growth conditions over an indeterminate, subregional scale. The two species tended to vary in the same fashion both among and within years, despite the offset of about 1 mo in their developmental schedules. Length of *N. plumchrus* was negatively correlated with Emerson's (1987. J. Geophys. Res. (Oceans) 92(C6): 6535–6544) estimates of May–August new production at Station P from 1969 to 1978.

La longueur du prosoma des stades dormants de *Neocalanus plumchrus* et de *Neocalanus flemingeri* a varié de façon significative, quoique modérément, d'une année à l'autre à la station P située dans le golfe d'Alaska. L'écart de la variation pendant 20 ans était faible par rapport à la variation géographique connue. Le fait que les variations de la longueur inter-annuelles étaient nettement plus importantes que les variations intra-annuelles porte à croire à une variation semblable, d'une année à l'autre, des conditions de croissance à une échelle sous-régionale indéterminée. La variation chez les deux espèces avait la même tendance à l'échelle inter-annuelle et intra-annuelle, malgré le décalage d'environ 1 mo de leurs régimes de développement. La longueur de *N. plumchrus* était en corrélation négative avec les estimations d'Emerson (1987. J. Geophys. Res. (Oceans) 92(C6): 6535–6544) en ce qui concerne la nouvelle production de mai à août, entre 1969 et 1978, à la station P.

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Serial observations of zooplankton biomass at Ocean Station P are among the most frequently cited demonstrations of interannual variation in the productivity of oceanic ecosystems (Wickett 1967; Longhurst et al. 1972; Frost 1983; Wiebe et al. 1987; Parsons and Lalli 1988). The observations, unfortunately now ended, were collected from ships on patrol at Ocean Weather Station P at 50°N, 145°W in the Gulf of Alaska. Ecological results from oceanographic work at Station P have stimulated continuing investigation of pelagic ecology in the subarctic sector of the Pacific. Part of that work is the Subarctic Pacific Ecosystem Research ("SUPER") Program, which mounted six expeditions to the Gulf in the 1980's. In the course of those cruises, we noted substantial differences between years in the body size of the large grazing copepods dominant in the region. This stimulated us to return to samples from the weathership time series in order to characterize this different aspect of interannual variability as fully as possible. Realized growth over the entire life cycle in these animals is an index of production conditions different from, but possibly correlated with, total plankton standing stock. We asked whether the year-to-year changes in standing stock might be caused in part by variations in body size of the dominant plankters.

Zooplankton samples were collected from the weatherships at variable intervals from 1956 through 1980. Biomass and taxonomic analyses of vertical hauls from 150 m to the surface were reported by Fulton (1983). In addition, there was a series of vertical hauls from 1000 or 1200 m to the surface collected at irregular intervals from 1961 through 1980. Some of these deep samples were analyzed in various ways, but no results have been published. Examination in July 1989 of the extant samples from this series showed at least some remaining wet in unbroken jars from every year of the series. From those we have selected a set for examination of size variation in *Neocalanus plumchrus* (Marukawa) 1921 and *Neocalanus flemingeri* Miller 1988.

The subarctic Pacific has three, endemic species of the copepod genus *Neocalanus: N. plumchrus, N. flemingeri*, and *N. cristatus*. The univoltine life histories of these species include a resting phase spent in mesopelagic depths (Miller et al. 1984; Miller and Clemons 1988). Particularly for *N. plumchrus* and *N. flemingeri*, the resting phase is sharply separated from the active growth phase. Moreover, the middle

TABLE 1. Weathership time series of PL's for *N. plumchrus* C5. Mean square values (MS) allow one-way ANOVA for significance of variation of means between samples within years. A conservative table of *F*-ratios is given at the bottom of the table.

Year	Date (* of next yr)	Mean PL	SD	*N*	Year mean	Among MS Within MS *F* (df)
1961	22 Sept.	3.637	0.1212	63	3.695	0.1817
	8 Oct.	3.751	0.1078	49		0.0128
	20 Oct.	3.696	0.1083	60		14.2 (2, 169)
1962	19 Aut.	3.606	0.0854	70	3.619	0.0311
	27 Aug.	3.608	0.1066	69		0.0103 NS
	31 Oct.	3.644	0.1105	69		3.03 (2, 205)
1963	26 Sept.	3.683	0.1626	91	3.616	0.0689
	24 Dec.	3.606	0.1471	9		0.0257 NS
	7 Jan.*	3.558	0.1424	7		2.68 (2, 104)
1964	24 Aug.	3.571	0.0916	56	3.596	0.0306
	8 Sept.	3.603	0.0931	70		0.00831
	19 June	3.615	0.0888	68		3.67 (2, 191)
1965	8 Aug.	3.726	0.1463	74	3.615	0.661
	29 Sept.	3.583	0.1542	63		0.022
	18 Oct.	3.536	0.1447	58		30.0 (2, 192)
1966	24 Aug.	3.523	0.1288	72	3.614	0.445
	2 Sept.	3.652	0.1154	71		0.016
	10 Nov.	3.666	0.1345	72		27.8 (2, 212)
1967	25 July	3.813	0.1330	66	3.835	0.0319
	17 Oct.	3.837	0.1134	58		0.0143 NS
	30 Dec.	3.856	0.1108	72		2.23 (2, 193)
1968	18 June	3.603	0.1179	63	3.632	0.0197
	20 Aug.	3.628	0.1380	74		0.0164 NS
	9 Nov.	3.664	0.1046	8		1.20 (2, 142)
	29 Jan.*	3.655	0.0974	5	(not incl. in mean)	
1969	20 Aug.	3.906	0.1037	70	3.835	0.350
	26 Sept.	3.760	0.1124	61		0.011
	11 Nov.	3.839	0.0993	67		31.7 (2, 195)
1970	20 Aug.	3.747	0.1250	67	3.741	0.0215
	10 Sept.	3.755	0.1127	67		0.0146 NS
	14 Nov.	3.720	0.1250	65		1.47 (2, 196)
1971	30 Sept.	3.578	0.1022	67	3.589	0.1322
	15 Oct.	3.551	0.1027	71		0.01225
	4 Dec.	3.638	0.1269	63		10.8 (2, 198)
1972	8 Aug.	3.640	0.1247	70	3.669	0.1324
	3 Sept.	3.719	0.1154	69		0.0134
	12 Oct.	3.648	0.1074	72		9.85 (2, 208)
1973	19 Aug.	3.747	0.0993	67	3.733	0.225
	3 Oct.	3.669	0.0827	64		0.00948
	28 Dec.	3.784	0.1087	67		23.75 (2, 195)
1974	1 Sept.	3.677	0.1212	68	3.650	0.143
	1 Nov.	3.596	0.1080	66		0.0117
	20 Nov.	3.676	0.0947	71		12.2 (2, 205)
1975	26 Sept.	3.812	0.1172	69	3.808	0.244
	20 Oct.	3.873	0.1082	59		0.0138
	27 Dec.	3.739	0.1279	50		17.7 (2, 175)
1976	16 Sept.	3.695	0.1321	68	3.723	0.0410
	18 Oct.	3.741	0.1103	69		0.0145 NS
	4 Nov.	3.732	0.1183	67		2.82 (2, 201)
1977	23 Sept.	3.642	0.1204	66	3.670	0.1315
	8 Oct.	3.646	0.1011	67		0.01345
	8 Nov.	3.722	0.1253	65		9.78 (2, 195)

TABLE 1. (*Concluded*)

Year	Date (* of next yr)	Mean PL	SD	*N*	Year mean	Among MS Within MS *F* (df)
1978	15 June	3.627	0.1256	75	3.650	0.03942
	23 Aug.	3.674	0.1245	69		0.01334 NS
	12 Jan.*	3.648	0.0896	62		2.954 (2, 203)
1979	8 Aug.	3.542	0.1285	71	3.575	0.0837
	9 Nov.	3.571	0.1157	69		0.0151
	8 Dec.	3.611	0.1239	69		5.55 (2, 206)
1980	20 Aug.	3.818	0.1467	67	3.759	0.1395
	23 Sept.	3.750	0.1566	69		0.0236
	1 Jan.*	3.709	0.1629	27		5.91 (2, 160)

Significant F values (df = 2, 120; one-tailed):
P = 0.05 0.025 0.01 0.005 0.001
F = 3.27 4.20 5.74 7.19 11.9
7 of 20 yr without significant within date variation at $P = 0.05$

copepodite stages in which terminal length probably is determined are brief, about 3 wk (Miller and Nielson 1988), and are consistent in timing over large regions. *Neocalanus flemingeri* rests as the adult female, in which individual length is probably determined mostly by growing conditions during the fifth copepodite (C5) stage which is passed in late April and May. *Neocalanus plumchrus* rests as the C5, for which body length (although not final biomass) must be established during C3 and C4, which are passed in May and June (Miller and Nielson 1988). Thus, the distribution of individual length in the deep resting stocks of these species represents growing conditions during spring in a general, seasonally integrated sense. Growing conditions for copepods are primarily affected by temperature and food availability (M. E. Huntley and M. D. G. Lopez, in review). *Neocalanus cristatus* was not included in the study because many fewer samples had sufficient numbers of individuals.

Deep resting stocks can be examined from late July through early January. The degree of consistency in length from month to month will be a measure of the consistency of spring growing conditions over an alongstream subregion (of indeterminate scale) from which resting stocks were installed into the deep waters flowing beneath Station P. Correlation in interannual variations between the two species will represent the consistency of interannual variation in growing conditions between spring (*N. flemingeri*) and early summer (*N. plumchrus*). Strong correlation would indicate that growth conditions of given years are set by events affecting the whole growing season, for example by mixing of the preceding winter. Absence of correlation would indicate that conditions vary more rapidly with month-to-month differences in the near-surface habitat producing the interannual changes in realized growth of single species.

Ideally, length variation could be compared with variation in oceanographic variables and climatic conditions. Thus, large mean length might, for example, be found to correspond to high early spring levels of nitrate or to above-average insolation from March through May. Unfortunately, the data series from the weatherships are not sufficiently complete or in suitably accessible form to allow such comparisons at present. Some interesting conclusions can be drawn without them.

Methods

Samples were collected by vertical hauls, from 1000 or 1200 m to the surface, of 56-cm-diameter nets equipped with 351-μm mesh. Details of net construction and hauling were the same as for nets used in the 150–0 m series reported by Fulton (1983). Samples were preserved in 4% formalin. We selected samples from the set remaining at Pacific Biological Station in July 1989. We sought to get a single sample in each of three separate months from August through December of each year. Actual separation in days varied considerably (Table 1) according to samples available. In some cases we had to use samples from June (three), July (one), or January of the following calendar year (three) to fill our quota of three samples per year. The June and July samples of *N. plumchrus* were certainly not all in the resting phase and many individuals were probably captured near the surface. However, since prosome length is the variable measured, it was probably established by the molt to C5.

Specimens were sorted from each sample until sets of 64 (or a few more) each of C5 *N. plumchrus* and female *N. flemingeri* were in hand. Samples with fewer than 64 specimens were sorted completely. Most sample sizes were >64, but some were smaller. Prosome length (PL) (as illustrated in Miller 1988) was then measured and tabulated for all specimens using an ocular micrometer-equipped stereomicroscope. Measures were made to the nearest micrometre unit, or approximately 1 part in 55 (shortest *N. flemingeri* female, 2.78 mm) to 1 part in 83 (longest *N. plumchrus* C5, 4.20 mm). The total numbers of specimens measured were 3807 *N. plumchrus* C5 and 3523 *N. flemingeri* females.

Results

Prosome lengths of both species were normally distributed within expected sampling error (Fig. 1) in all but 3 of 56 samples with $N > 30$ of *N. plumchrus* (goodness-of-fit χ^2 gave $0.05 < P < 0.1$ for all three) and in all but 1 ($P = 0.05$) of 55 samples of *N. flemingeri* with $N > 30$. This is less than the expected fraction of cases deviating. There were no instances of multimodal distribution. Four distributions were apparently leptokurtic, but all analyses should be robust to that. Inspection

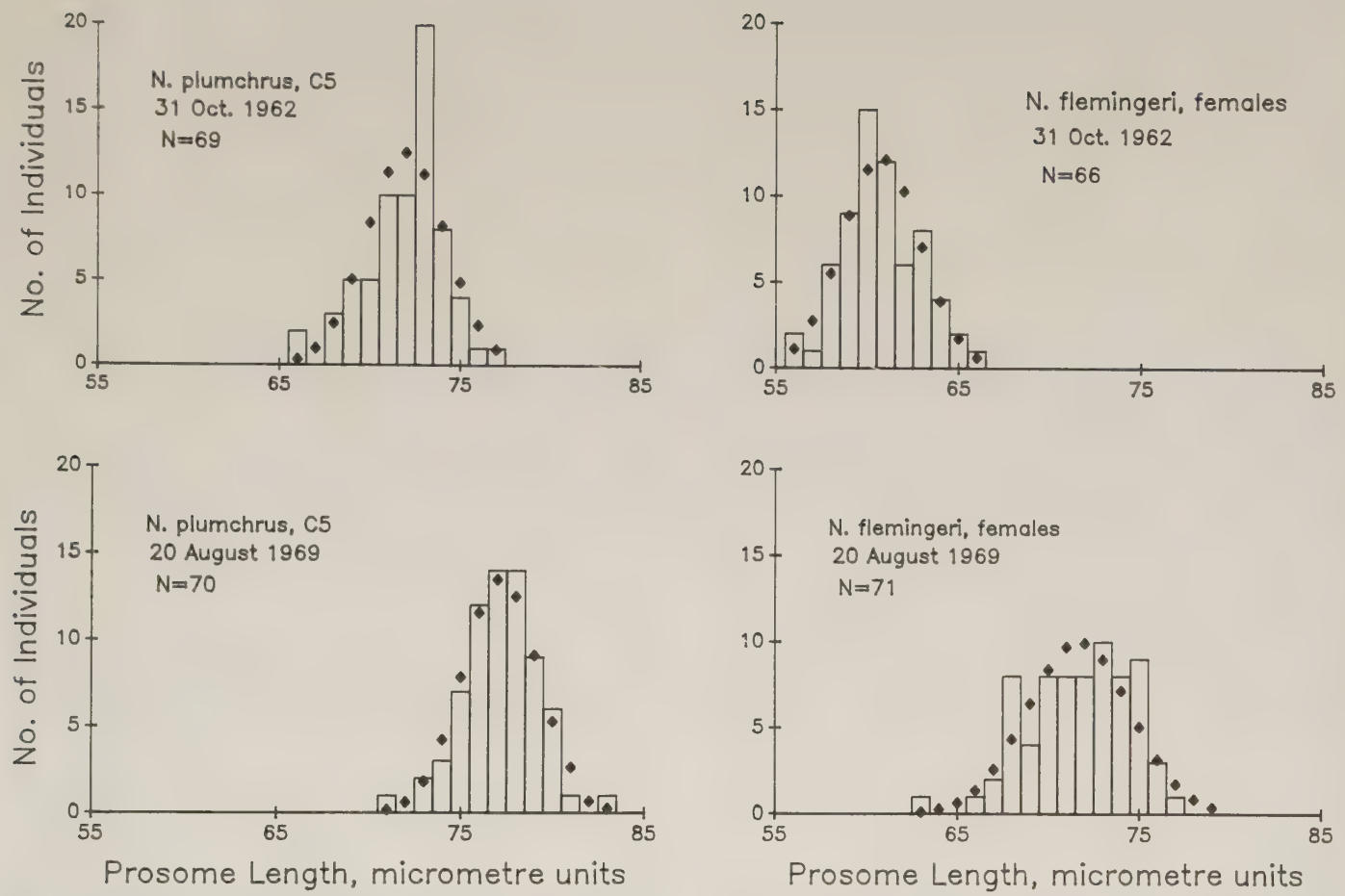

FIG. 1. Histograms showing length frequency distributions of the form typical in this study. Diamonds are the expected frequency in each length category for normal distributions fitted from the observed mean and standard deviations. Smallest mean lengths were observed on 31 October 1962 (pair above). Largest mean lengths were observed on 20 August 1969 (pair below). Goodness-of-fit χ^2 values for this set of four ranged from 9.61 (6 df, $P < 0.1$) for *N. plumchrus* on 31 October 1962 to 0.49 (6 df) for *N. plumchrus* on 20 August 1969.

of the standard deviations (Tables 1 and 2) shows close similarity throughout the data set. There were almost no pairs of samples with significant F ratios. Thus, the assumption of equal variances in our analyses of variance (ANOVA) is met to within sampling errors.

Between-year variation of PL in both species was significantly greater than the within-year variation (Fig. 2). This is established by two-level, nested ANOVA's with corrections for unequal sample sizes (Sokal and Rohlf 1981, our analysis is comparable with their Box 10.4, p. 294). For both *N. flemingeri* females (Table 3) and *N. plumchrus* C5 (Table 4), the among-sample and among-year F values are significant at $P < 0.001$. The variance components are as follows:

	N. flemingeri		*N. plumchrus*	
Among years	0.01410	47%	0.00592	26%
Within years	0.00188	6.3%	0.00234	10%
Within sample	0.01391	47%	0.01423	63%

That agrees with the obviously greater year-to-year variation of *N. flemingeri*. The within-sample variances (pooled over all samples) are very close to the squares of the mean sample standard deviations (0.01404 for *N. flemingeri* and 0.01405 for *N. plumchrus*), again implying that the sample variances are quite homogeneous.

There was some, but not excellent, agreement between the species as to which years had large and which small specimens. This is seen by inspection of Fig. 2. Not all features are reproduced in the two time series, but the peaks of 1967, 1969, 1973, and 1980 and the low point of 1979 occur in both. The agreement is shown by the correlation coefficient, $r = 0.687$ (for $P = 0.01$, $r = 0.561$), of the annual means (unweighted means of the three sample means) and by the scatter diagram (Fig. 3).

While length for both species differed significantly between years (because within-year variation was less than between-year variation), there was still significant within-year variation. By single-factor ANOVA's (Tables 1 and 2) this was shown to be significant for somewhat more than half the years. Moreover, the two species agreed to some extent about this between-sample variation. If the three samples from each year are ranked for each species and the absolute value of the differences of the ranks (|*flem* rank − *plum* rank|) are summed over the samples and the samples over the years, the result is 26. That compares with a rough expectation of 53.3 (only even integral values are possible), with probability of a sum of 26 or smaller and of sums as improbably large of 0.0001 (calculated from all possible combinations of ranks of 20 paired sets of three values). This agreement does not increase the between-species correlation based on all samples ($r = 0.662$) compared with that of the annual means ($r = 0.687$; Fig. 4).

TABLE 2. Weathership time series of PL's for *N. flemingeri* females. Mean square values (MS) allow one-way ANOVA for significance of variation of means between samples within years. A conservative table of *F*-ratios is given at the bottom of the table.

Year	Date (* of next yr)	Mean PL	SD	*N*	Year mean	Among MS Within MS *F* (df)
1961	22 Sept.	3.284	0.1323	35	3.332	0.1537
	8 Oct.	3.394	0.1095	57		0.0133
	20 Oct.	3.317	0.110	57		11.6 (2, 146)
1962	19 Aug.	3.024	0.1204	34	3.043	0.0400
	27 Aug.	3.030	0.0975	43		0.117
	31 Oct.	3.075	0.1083	66		3.42 (2, 140)
1963	26 Sept.	3.297	0.1146	64	3.295	0.2485
	24 Dec.	3.360	0.1338	87		0.0153
	7 Jan.*	3.229	0.1152	41		16.2 (2, 189)
1964	24 Aug.	3.235	0.1168	19	3.241	
	8 Sept.	3.246	0.1269	25		
	19 June	3.003	0.1057	69	(C5's not incl. in mean)	
1965	8 Aug.	3.440	0.1124	60	3.425	0.1318
	29 Sept.	3.429	0.1106	53		0.01359 NS
	18 Oct.	3.407	0.1298	40		0.970 (2, 150)
1966	24 Aug.	3.135	0.1353	50	3.214	0.1600
	2 Sept.	3.232	0.1128	69		0.01525
	10 Nov.	3.257	0.1250	62		10.5 (2, 178)
1967	25 July	3.454	0.1385	71	3.447	0.0426
	17 Oct.	3.417	0.1418	50		0.0203 NS
	30 Dec.	3.471	0.1473	65		2.10 (2, 183)
1968	18 June	3.097	0.1420	29	3.180	
	20 Aug.	3.256	0.1408	13	(*N*-weighted mean)	
	9 Nov.	3.392	—	1		
	29 Jan.*	3.301	0.1467	10		
1969	20 Aug.	3.629	0.1431	71	3.573	0.247
	26 Sept.	3.512	0.1273	72		0.0182
	11 Nov.	3.578	0.1335	70		13.6 (2, 210)
1970	20 Aug.	3.279	0.1193	70	3.304	0.0477
	10 Sept.	3.331	0.1159	68		0.136
	14 Nov.	3.303	0.1140	48		3.50 (2, 183)
1971	30 Sept.	3.578	0.1022	67	3.589	0.1322
	15 Oct.	3.551	0.1027	71		0.01225
	4 Dec.	3.638	0.1269	63		10.8 (2, 198)
1972	8 Aug.	3.640	0.1247	70	3.669	0.1324
	3 Sept.	3.719	0.1154	69		0.0134
	12 Oct.	3.648	0.1074	72		9.85 (2, 208)
1973	19 Aug.	3.747	0.0993	67	3.733	0.225
	3 Oct.	3.669	0.0827	64		0.00948
	28 Dec.	3.784	0.1087	67		23.75 (2, 195)
1974	1 Sept.	3.677	0.1212	68	3.650	0.143
	1 Nov.	3.596	0.1080	66		0.0117
	20 Nov.	3.676	0.0947	71		12.2 (2, 205)
1975	26 Sept.	3.812	0.1172	69	3.808	0.244
	20 Oct.	3.873	0.1082	59		0.0138
	27 Dec.	3.739	0.1279	50		17.7 (2, 175)
1976	16 Sept.	3.695	0.1321	68	3.723	0.0410
	18 Oct.	3.741	0.1103	69		0.0145 NS
	4 Nov.	3.732	0.1183	67		2.82 (2, 201)
1977	23 Sept.	3.642	0.1204	66	3.670	0.1315
	8 Oct.	3.646	0.1011	67		0.01345
	8 Nov.	3.722	0.1253	65		9.78 (2, 195)

TABLE 2. (*Concluded*)

Year	Date (* of next yr)	Mean PL	SD	*N*	Year mean	Among MS Within MS *F* (df)
1978	15 June	3.627	0.1256	75	3.650	0.03942
	23 Aug.	3.674	0.1245	69		0.01334 NS
	12 Jan.*	3.648	0.0896	62		2.954 (2, 203)
1979	8 Aug.	3.542	0.1285	71	3.575	0.0837
	9 Nov.	3.571	0.1157	69		0.0151
	8 Dec.	3.611	0.1239	69		5.55 (2, 206)
1980	20 Aug.	3.818	0.1467	67	3.759	0.1395
	23 Sept.	3.750	0.1566	69		0.0236
	1 Jan.*	3.709	0.1629	27		5.91 (2, 160)

Significant F values (df = 2, 120; one-tailed):

P =	0.05	0.025	0.01	0.005	0.001
F =	3.27	4.20	5.74	7.19	11.9

7 of 20 yr without significant within date variation at $P = 0.05$

TABLE 3. ANOVA table for PL of *N. flemingeri* females at Station P from 1961 to 1980.

Source	df	Sum of squares	Mean square	*F*	*F'*[a]
Among years	19	49.406	2.600	20.65	20.47
Among samples	39	4.910	0.1259	9.75	
Within sample	3463	48.176	0.01391		
Total	3521	102.492	$F[P = 0.001, 19, 39] = 3.23$		

[a]*F' calculated with MS' (among samples) calculated with Satterthwaite's correction for unequal sample sizes.*

TABLE 4. ANOVA table for PL of *N. plumchrus* C5 at Station P from 1961 to 1980.

Source	df	Sum of squares	Mean square	*F*	*F'*[a]
Among years	19	24.516	1.2903	8.16	7.56
Among samples	40	6.324	0.1581	11.11	
Within sample	3742	53.242	0.01423		
Total	3801		$F[P = 0.001, 19, 40] = 3.20$		

[a]*F' calculated with MS' (among samples) calculated with Satterthwaite's correction for unequal sample sizes.*

Discussion

Between-year variation in length of resting stage *N. plumchrus* and *N. flemingeri* is greater than within-year variation. That is evidence of homogeneity of development conditions over a moderately large part of the southern subarctic Pacific during the spring – early summer growth season in any given year. Since the samples from depth are typically separated by months, the surface source waters of the deep stocks represented must have been substantially separated horizontally. As is common in oceanographic practice, we cannot say by how much they were separated, but the resulting habitat variation does not produce as much variation in length as the interannual difference. Taking a grand mean, geostrophic velocity for surface waters west of Station P is on the order of 4.3 km·d^{-1} (Favorite et al. 1976); so (assuming slower currents at depth), the maximum separation of source sites would have been considerably less than 1000 km. Given this moderate

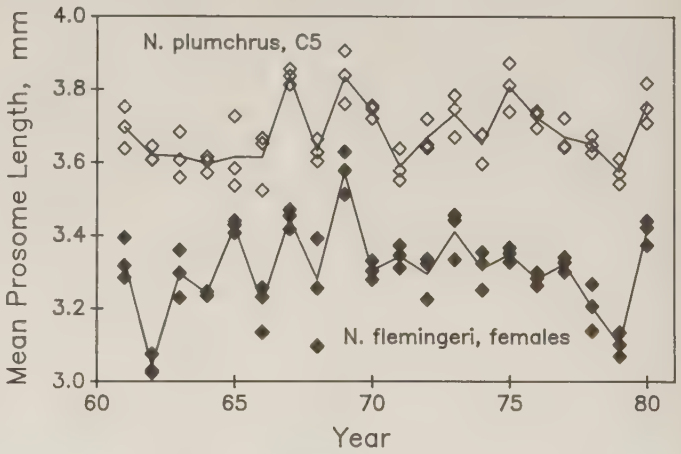

FIG. 2. Time series of mean lengths for *N. plumchrus* C5 (open symbols) and *N. flemingeri* females (solid symbols). Each symbol is the mean for a single sample. The overall means for the years (unweighted mean of the sample means) are connected by lines.

advection, the observed within-year agreement is not particularly surprising.

Rough agreement betwen the species as to years in which they are large or small implies that, despite the usual difference in the timing of their development, the species are affected similarly by differences in "growth potential" of different years. Growth potential must usually be established by April – early May, when *N. flemingeri* is in C5 establishing PL for C6, and remain about the same (relative to the difference among years) through June, when *N. plumchrus* completes C4 establishing PL for C5. As stated above, there must also be considerable regional homogeneity. This is like the alongstream coherence in timing of life cycle events demonstrated by Miller et al. (1984). April–May is the period in which the upper water column restratifies in the subarctic Pacific. Thus, the greater interannual variability of *N. flemingeri* lengths may derive from the effect of variations in restratification timing on copepod food supplies.

Along with this regional homogeneity, there is nevertheless a degree of spatial variation in growth conditions. This is implied by the statistical significance of within-year variation of both species (Tables 3 and 4). Thus, while there is evidence

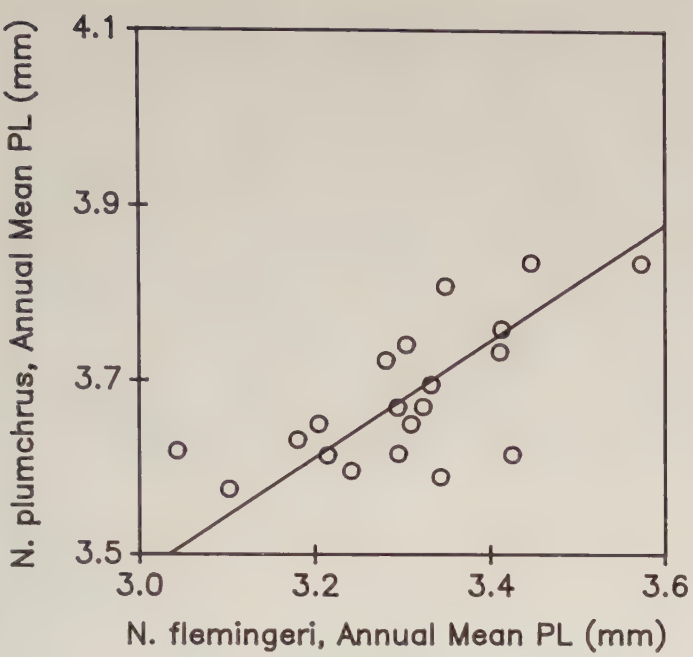

FIG. 3. Comparison of annual mean (unweighted mean of sample means) PL for *N. plumchrus* C5 and *N. flemingeri* females. The greater overall length variability of the smaller *N. flemingeri* is shown by the clustering of points in the lower part of the graph. The fitted line is a principal axis of the data, appropriate to represent the relationship of two variates measured in the same units and roughly equal variance at each point (Sokal and Rohlf 1981).

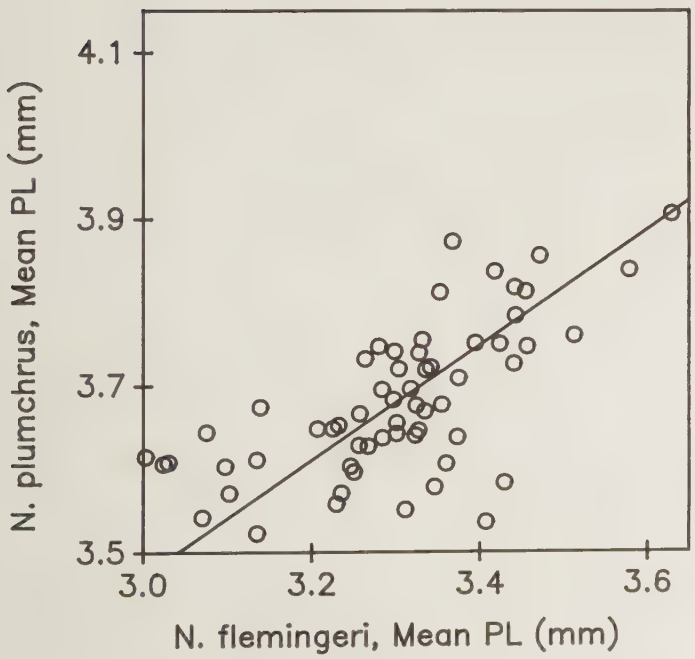

FIG. 4. Comparison of all sample means of PL for *N. plumchrus* C5 and *N. flemingeri* females. The fitted line is a principal axis. The relationship is neither stronger nor weaker than that of annual means (Fig. 3).

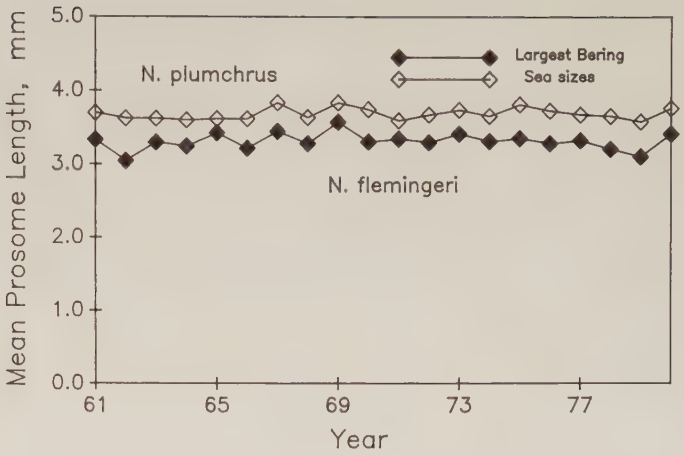

FIG. 5. Annual means from Fig. 2 rescaled to show interannual length variation relative to total length. Lengths of the largest *N. plumchrus* C5 (solid symbols) and *N. flemingeri* females observed in Bering Sea collections (Miller 1988) are shown by the lines at the top. While *N. flemingeri* is smaller in all stages at Station P, it can be the larger species in sufficiently cold, food-rich waters.

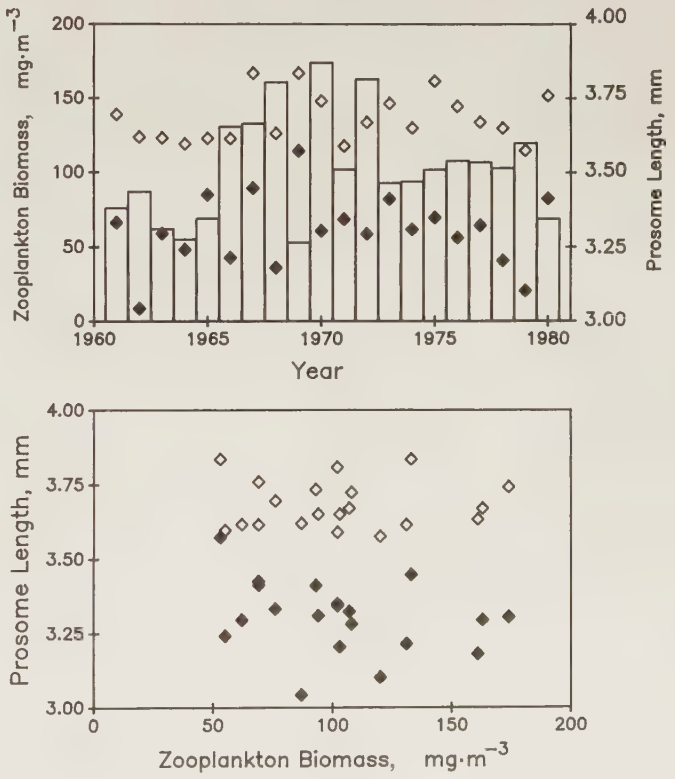

FIG. 6. Comparison of annual mean biomass estimates taken from Frost's (1983) analysis of Fulton's (1983) compilation of 150–0 m net haul data from Ocean Station P. Top: zooplankton biomass as vertical bars (left scale); annual mean PL of *N. plumchrus* C5 and *N. flemingeri* females as open and solid symbols, respectively (right scale). Bottom: Scatter diagram of annual mean length against the annual mean biomass.

of regional homogeneity in development conditions, residual variability is enough that resting stocks do show significant length variation. It is a curious wrinkle in this comparison that there is also significant agreement between the resting populations of the two species in the difference between sampling

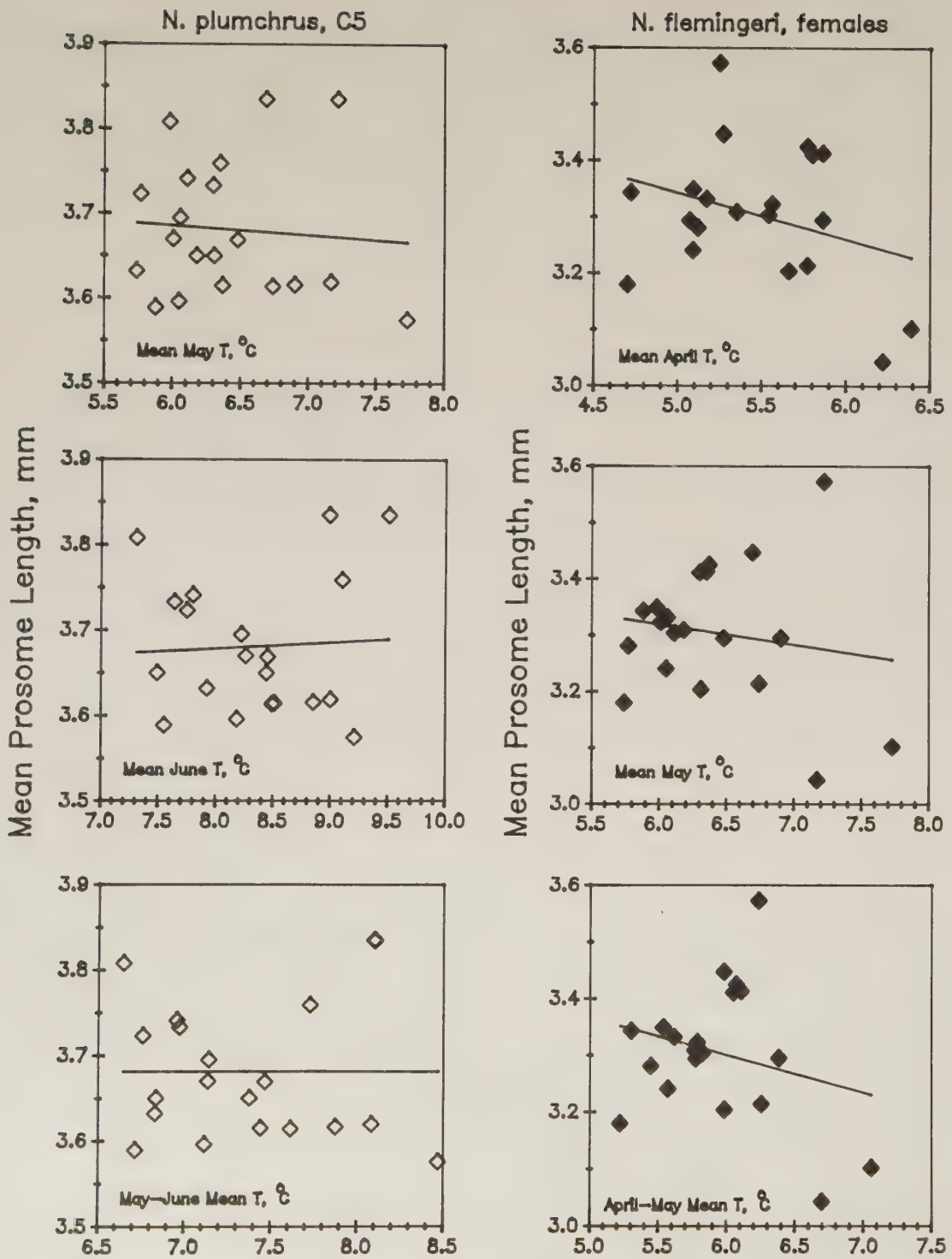

FIG. 7. Scatter diagrams of annual mean resting stage prosome length for *N. plumchrus* C5 and *N. flemingeri* females versus monthly mean temperatures of the growth year. May and June were chosen for *N. plumchrus* because middle copepodite stages are passed in May and C4 and C5 in June. April and May were chosen for *N. flemingeri* for the same reason. All regressions were nonsignificant.

dates in respect to mean length. Apparently, the female *N. flemingeri* and C5 *N. plumchrus* that are found mixed together at depth grew up in conditions similar enough, albeit up to 1 mo apart (Miller and Clemons 1988), to have been similarly affected in their length. The spatial separation of waters producing stocks in different deep samples appears to have been greater than the spatial and ecological separation of waters producing the resting stocks of the two species collected together at depth. There probably is no means for quantifying this deduction.

We can see year-to-year variation of length at Station P very clearly (Fig. 2) because of the quantity of our data, but it is small relative to mean body length and small relative to variation over the species range as a whole (Miller 1988). The variation of length is better represented in Fig. 5, scaled from 0 to 5.0 mm and showing the lengths of the largest specimens

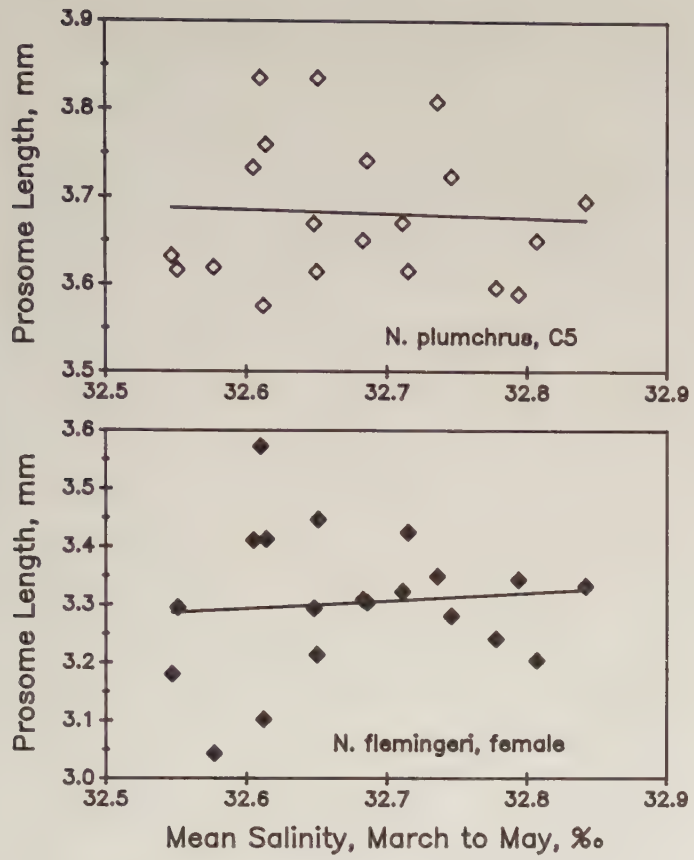

FIG. 8. Scatter diagrams of annual mean resting stage PL for *N. plumchrus* C5 and *N. flemingeri* females versus the mean salinity for March (some February data were substituted) to May of the growth year. There is no relationship.

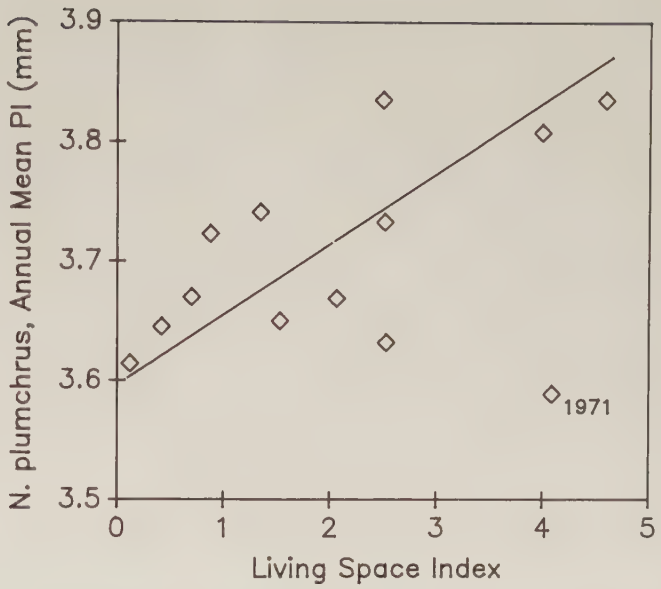

FIG. 9. Annual mean resting stage PL of *N. plumchrus* plotted against a "living space index" for 1965–77 only. The index is the mean April–June upward Ekman transport divided by mean June–July copepod abundance (number·m^{-3}). Fitted line is a geometric mean regression appropriate to variates with different units of measure, both with substantial variance (Sokal and Rohlf 1981). The line ($r = 0.74$, $P < 0.01$) was fit after removal of the 1971 point. There is no basis for excluding this point; when it is included, correlation drops to $r = 0.46$ (not significant).

from the Bering Sea, than in Fig. 2, scaled from 3.0 to 4.0 mm. Of course, there is a larger impact on body volume (proportional to the cube of length), but even that is not impressive. As suggested by Dagg and Walser (1987) on other grounds, the Gulf of Alaska is always marginal for feeding by these species. This is a partial explanation for the short specimens at Station P. The warmer temperatures of the Gulf of Alaska, compared with the Bering Sea and western subarctic, also would lead to shorter specimens. Although modest, the observed size variation is enough to be ecologically significant. For example, Fulton (1973) showed a rough correlation between PL and total egg production by *N. plumchrus* females from the Strait of Georgia. Assuming that size differences among C5's translate to differences among females after maturation, and applying the Strait of Georgia regression, the maximum difference we observed in annual means (between 3.575 mm in 1979 and 3.835 mm in 1967 and 1969) would correspond to a difference of 25% in total egg production. Such differences would produce some carryover of population processes between years.

How much of the strong interannual variation in zooplankton biomass at Station P (Fulton 1983; Frost 1983) is attributable to differences in individual copepod size? The small absolute variation in size (Fig. 5) suggests that it cannot be much. Moreover, we found no evidence in this data set (Fig. 6) that year-to-year zooplankton biomass variation is even slightly correlated with differences in individual size of the dominant copepods. Individual length responds independently from total zooplankton biomass to variations in growing conditions in the Gulf of Alaska.

We have examined the correlation of our PL time series with habitat variables. Some desirable variables (year-to-year mixing depth variation) are not catalogued in readily accessible form; some (nutrients) were too sparsely sampled to be useful. Dr. S. Tabata has graciously provided us with listings of monthly mean temperature and salinity data collected from the weatherships that are the basis of his papers on oceanographic variability at Station P (Tabata 1989, 1990). Growing season temperature might be expected to be negatively correlated with terminal size, based on experience with coastal copepods (Deevey 1960; Landry 1978; Corkett and McLaren 1978; Miller 1983). However, while mean temperature during the months in which copepodite growth occurs varied about 2°C among years, there is no correlation with subsequent resting stage size (Fig. 7).

High early spring salinity may be correlated with strong or deep winter mixing, and thus might be expected to be associated with higher nutrients and larger body size. Salinity is strongly correlated among months in the spring period, but differs by 0.3‰ among years. Therefore, correlation was examined between resting stage PL and the mean of March–June salinity (Fig. 8). There was none.

Examination of scatter plots of lengths against various sums of monthly vertical Ekman transport for 1965–77 (Ballantyne and Wicket 1978) showed no apparent correlations. An elaborate "index of living space," calculated as (mean April–June W_{Ekman})/(June–July copepods·m^{-3}), was weakly correlated with PL in *N. plumchrus* (Fig. 9). The idea is that if Ekman transport is related to nutrient supply, and thus food, and animal density reflects competition, this index should express their interaction. The correlation may or may not be meaningful. No

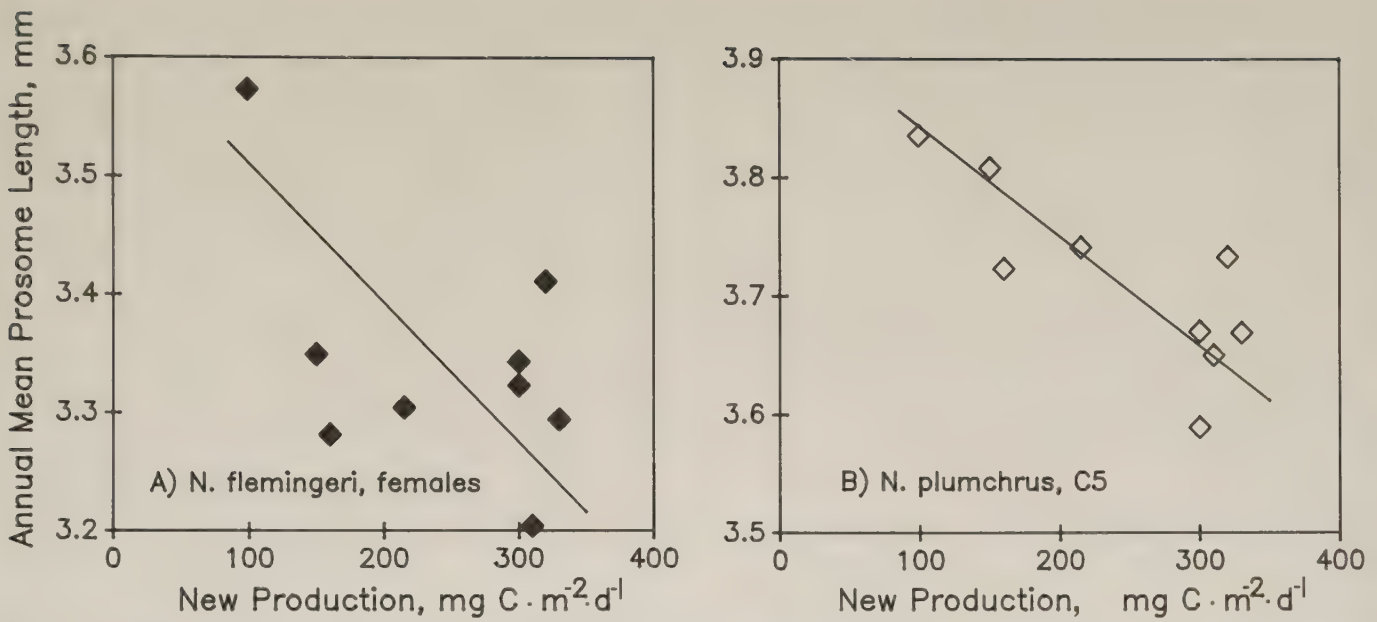

FIG. 10. Comparison of mean PL's of (A) *N. flemingeri* females and (B) *N. plumchrus* C5 with mid-May to mid-August "new production" estimates for 1969–78 from Emerson (1987). Fitted lines are geometric mean regression lines. For *N. flemingeri* the correlation coefficient of -0.51 is not significant. For *N. plumchrus* the correlation coefficient is -0.81, and $P < 0.01$.

other combination of transport variables showed any correlation.

Emerson (1987) has used Ocean Station P oxygen data to generate a 9-yr series of "new production" estimates for mid-May to mid-August, the annual period in which oxygen supersaturation results from (1) mixed layer warming, (2) photosynthesis exceeding respiration, and (3) reduced winds to reduce gas exchange at the sea surface. Estimates of effects of warming and gas exchange rate on supersaturation allowed calculation of the photosynthetic excess, which Emerson expressed as $(P-R)$. The $(P-R)$ values were converted to a carbon-based new production estimate by multiplying by a Redfield ratio, C/O = 138/106. Our annual mean PL's for *N. flemingeri* were negatively, but nonsignificantly, correlated with these oxygen-based, new production estimates (Fig. 10A). *Neocalanus plumchrus* PL's were negatively and strongly correlated (Fig. 10B).

The result for *N. plumchrus* is counterintuitive. More new production should correlate with more total primary production, which should produce more copepod food, leading to greater body size. However, Emerson's new production variate is neither production based on nutrients newly supplied from outside the euphotic zone (as NO_3^-, or perhaps soluble iron) nor production that is necessarily exportable in the long term. It is simply a difference over a roughly 5- to 15-d gas equilibration time betwen photosynthetic oxygen (and organic matter) production and respiratory oxygen (and organic matter) consumption. It can vary either because (1) total production varies and respiration removes a nearly constant fraction or (2) respiration consumes a variable fraction. The negative correlation of *N. plumchrus* PL with $(P-R)$ suggests the latter explanation. Most herbivory in the oceanic, subarctic Pacific is by microzooplankton (Miller and SUPER Group 1988), probably by a microbial food web of some complexity. The *Neocalanus* species feed on the output of this food web. If it is more complex, it will respire more of the primary production (R will be large and $P-R$ small), but possibly the transfer to *Neocalanus* would be by larger, more nutritious microzooplankton. Thus, the value of $(P-R)$ may depend mostly on variation in R driven by a changing average number of trophic steps in the microbial food web.

This explanation is attractive in another respect. If R/P were roughly constant, then interannual variation in $(P-R)$ amounting to a factor as large as 3.4 would require that P also vary by a factor of 3.4 between years. That is a much larger difference than is likely from variation in insolation or from observed variation of phytoplankton stock. It is possible that variation in a limiting micronutrient, such as iron (Martin et al. 1989), could produce so great a range in P, since interannual variation in supply over the North Pacific is at least that great (Duce 1986). A test between the "iron limitation" and "variable R" hypotheses is that iron limitation should produce a huge variation in total annual production (new and regenerated), while variable R would not. We need time series data in the subarctic Pacific adequate to address the variation in annual total primary production.

Since PL for *N. flemingeri* is mostly established before the mid-May start of Emerson's (1987) season of estimation for $(P-R)$, it is not very surprising that it shows less correlation (Fig. 10A). Similar correlation with $(P-R)$ is expected, since PL's of the two species are positively correlated with each other. However, all of these correlations are loose enough to allow significance for some variate pairs and not for others.

The PL measures with which we had to represent individual size in the long-preserved deep samples are not a measure of individual biomass, since large variations are possible in the relationship of length to weight. However, they do represent conditions for growth during the middle copepodite stages in which body length is established for the resting stages. Those conditions must vary significantly (Fig. 2), but modestly (Fig. 5), among years. Changes in mean length could result from variations in temperature or food availability, or both, during growth prior to the molt producing the resting stage. Food availability for *Neocalanus* species may vary because of

variation in total system productivity or because of shifts in the character of the microzooplankton food web.

Acknowledgements

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Dynamic Model of the Flux of Domoic Acid, a Neurotoxin, through a *Mytilus edulis* Population

W. Silvert and D. V. Subba Rao

Department of Fisheries and Oceans, Habitat Ecology Division, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, N.S. B2Y 4A2, Canada

Silvert, W., and D. V. Subba Rao. 1992. Dynamic model of the flux of domoic acid, a neurotoxin, through a *Mytilus edulis* population. Can. J. Fish. Aquat. Sci. 49: 400–405.

A one-compartment computer simulation model was developed to quantify and characterize the accumulation of the neurotoxin domoic acid in blue mussel (*Mytilus edulis*) in Cardigan Bay, Prince Edward Island, Canada. Comparison of simulation results with field abundances of *Nitzschia pungens* f. *multiseries* Hasle, a diatom implicated in the production of domoic acid, and with measurements of domoic acid within *Nitzschia* cells indicates that the rate of accumulation of domoic acid in mussel tissue during peak bloom conditions may involve unexpected physiological feedback processes. At extremely high concentrations of toxin (>300 ppm) observed in toxic *Mytilus* episodes off eastern Prince Edward Island in 1987 during the peak of *Nitzschia* bloom, the depuration of domoic acid seemed to be suppressed; this may have been responsible for the observed abnormal buildup of toxin in the mussels. In 1989 the amount of domoic acid produced by *N. pungens* was slightly lower, but the levels of domoic acid in *Mytilus* were much less than in 1987. These results suggest that a crucial factor in prediction of high levels of domoic acid in mussels may be identification of changes in their physiology and metabolism which suppress depuration rates.

Les auteurs ont élaboré un modèle de simulation informatisé à compartiment unique pour quantifier et caractériser l'accumulation d'acide domoïque, une neurotoxine, chez la moule bleue (*Mytilus edulis*) de la baie Cardigan, Île-du-Prince-Édouard. Une comparison des résultats ainsi obtenus avec l'abondance naturelle de *Nitzschia pungens* f. *multiseries* Hasle, une diatomée jouant un rôle dans la production d'acide domoïque, et des quantifications des teneurs en acide domoïque de cellules de *Nitzschia* a révélé que le taux d'accumulation de cet acide dans les tissus de la moule lors des périodes de pointe des poussées phytoplanctoniques peut être le résultat de processus imprévus de rétro-action physiologique. Aux concentrations très élevées de toxine (>300 ppm) observées en 1987 chez des *Mytilus* toxiques lors du maximum de la poussée phytoplanctonique de *Nitzschia* dans les eaux côtières de l'est de l'Île-du-Prince-Édouard, la fonction de dépuration de l'acide domoïque semblait avoir été inhibée. Ceci peut être à l'origine de l'accumulation anormale de toxine observée chez les moules. En 1989, la quantité d'acide domoïque produite par cette diatomée était légèrement moins élevée, mais les teneurs retrouvées chez la moule bleue étaient nettement inférieures à celles notées en 1987. Les résultats obtenus portent à croire que l'identification des variations physiologiques et métaboliques entraînant la suppression de la function de dépuration chez la moule pourrait être un facteur critique pour la prévision de teneurs élevées en acide domoïque chez cet organisme.

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During early winter 1987, a novel shellfish poisoning caused by the neurotoxin domoic acid adversely affected the blue mussel (*Mytilus edulis*) culture industry in Cardigan Bay, eastern Prince Edward Island, Canada (46°10′N, 62°30′W) (Addison and Stewart 1989). This toxin episode recurred early in the winter season in 1988 and 1989 and spread to adjacent bays. The macroalga *Chondria baileyana* Montagne, an archived sample of which yielded some domoic acid, and the pennate diatoms *Amphora coffaeformis* Kützing and *Nitzschia pungens* f. *multiseries* Hasle were considered as possible sources of domoic acid, which is an amino acid. Of these, *Chondria* and *Amphora* were rejected, as they were judged not to occur in sufficient quantities to explain the levels of domoic acid in the mussels. However, blooms of *N. pungens* f. *multiseries* in concentrations approaching 10^7 cells/L during October–December (Subba Rao et al. 1988a, 1988b) constitute a major food source of mussels, so *Nitzschia* was an obvious suspect. Laboratory cultures of *Nitzschia* yielded domoic acid (Subba Rao et al. 1988a, 1988b, 1990) as did the virtually monospecific blooms of *Nitzschia* from Cardigan Bay (Bates et al. 1989). Approximately 500 t of mussels are produced annually in Cardigan Bay; the commercial product is about 350 t (Addison and Stewart 1989), and we estimate that wild populations and unmarketable cultured mussels account for another 150 t. Domoic acid levels in mussels were as high as ~300 μg/g wet weight of mussel tissue (referred to as ppm hereafter), which is equivalent to 150 kg of acid in 500 t of shellfish. Whether *Nitzschia* can supply enough domoic acid to account for these high concentrations has not been established. To improve our quantitative understanding of the processes that lead to the accumulation of domoic acid, we developed and describe here a single-compartment model of the domoic acid flux through *M. edulis*.

Data Sources and Experimental Results

Nitzschia abundance data were taken from Judson and Bernard (1988), Hanic (1989), Bates et al. (1989), and Smith

et al. (1990a, 1990b). Mussel harvest and domoic acid levels were taken from Judson and Bernard (1988), Hanic (1989), Bates et al. (1989), and L. Lea (pers. comm. based on Department of Fisheries and Oceans, P.E.I. area, records). Domoic acid levels in *Nitzschia* are given in Subba Rao et al. (1988a, 1988b, 1990), Bates et al. (1989), and Smith et al. (1990a, 1990b).

The *Nitzschia–Mytilus* toxin episode during 1988 lasted about 100 d (Bates et al. 1989; Smith et al. 1990a). From early October the algal abundance steadily increased to a maximum of 1.2×10^6 cells/L by mid-November, followed by an equally rapid decline to the original levels by the end of the year (see Fig. 2a). There was no measurable cellular domoic acid in the early stages of the bloom, but the levels showed a steady buildup to about 6 pg/cell by late October and reached the maximum of 7 pg/cell by the beginning of December, i.e. 10 d after the peak abundance of *Nitzschia*. This temporal pattern is consistent with earlier observations on cultures (Subba Rao et al. 1990). Subsequently the cellular domoic acid decreased to undetectable levels by the end of the year. The total toxin bound in *Nitzschia* followed the same pattern as the algal abundance, with their peaks coinciding in mid-November (see Fig. 2b). However, domoic acid in the mussels was extremely low up to this time and then exponentially increased to attain the maximum of 300 ppm at the beginning of December, namely 15 d after the *Nitzschia* peak or 5 d after the peak in cellular domoic acid (see Fig. 4).

During the 1989 season the situation was very different. Even though there was a comparable amount of particulate domoic acid available to the mussels, the amount actually detected in *Mytilus* was far less than in the preceding season (Smith et al. 1990b).

Filtration rates of *Mytilus* were determined from several grazing experiments carried out in collaboration with Gary Wohlgeschaffen of Dalhousie University. In each experiment, 10 *Mytilus* from Cardigan Bay, 41.3–43.5 mm long with individual wet weights between 4.25 and 7.30 g, were set in a plastic mesh cage immersed in 10 L of *Nitzschia* culture held at 10°C. The algae were kept suspended by bubbling air through the culture, and light levels were kept low (9 $\mu E \cdot m^{-2} \cdot s^{-1}$) to inhibit growth. Initial levels of cell numbers and chlorophyll were determined. Hourly samples for cell numbers and chlorophylls were taken. The cage was then transferred to fresh culture medium. The experiment was terminated after 6 h. Wet and dry weights (constant after 24 h at 60°C) of the mussels were determined. Cell numbers were determined following Utermohl's sedimentation method using a Nikon microscope. Chlorophyll *a* was measured on 50-mL samples in duplicate using the fluorometric method of Holm-Hansen et al. (1965).

The initial concentrations of *Nitzschia* in our experiments on grazing by *Mytilus* ranged from 0.52×10^6 to 11.64×10^6 cells/L. Chlorophyll *a* levels ranged between 0.70 and 20.53 μg/L. Both cell numbers and chlorophyll levels are comparable with fall–winter blooms of *Nitzschia* observed in Cardigan Bay during the 1988 and 1989 blooms and thus reflect the natural situation. The grazing rate experiments indicate that at these concentrations the filtration rate of mussels of the average size found in Cardigan Bay (6.5 g) would be approximately 40 $L \cdot mussel^{-1} \cdot d^{-1}$. The filtering rate actually varies with cell concentration, as discussed later (G. Wohlgeschaffen, pers. comm.).

In analyzing the grazing experiments, we assume that the change in the number of cells results entirely from grazing, so that

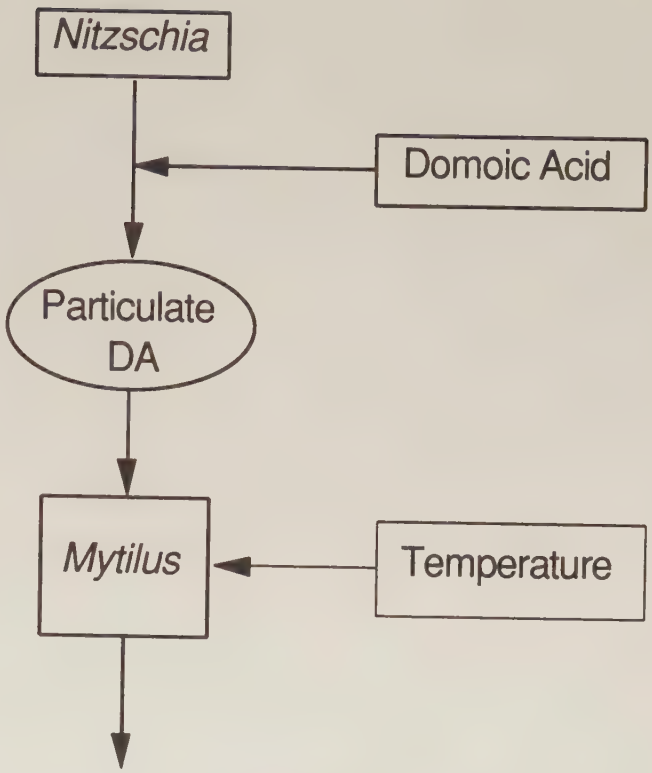

FIG. 1. Flow diagram for *Mytilus* model.

$$dA/dt = -N \cdot F \cdot A/V$$

where V is the volume of water, A is the concentration of algal cells at time t, N is the number of mussels, and F is the filtering rate (Coughlan 1969). Additional changes due to settling and algal growth are considered small and are ignored.

Modelling

To describe and quantify the dynamics of this system, we developed a model (Fig. 1) which represents the uptake of domoic acid through the ingestion of *N. pungens* containing a known concentration of domoic acid, and the subsequent depuration from *M. edulis*. Both the uptake and depuration processes are assumed to be temperature dependent. This model incorporates assumptions commonly made about the dynamics of domoic acid in the literature and in unpublished communications from researchers in the field. It is generally agreed that virtually all of the domoic acid ingested passes directly through the gut, and consequently there is no a priori need to introduce more complicated multicompartmental models to represent the retention of domoic acid in other organs. *Mytilus* ingests domoic acid only in particulate form and does not assimilate dissolved acid; excretion of domoic acid is observed only in very old cultures (G. Wohlgeschaffen, pers. comm.; Novaczek, unpublished). The model was intended to test these assumptions and to determine whether they agreed quantitatively with the results of field and laboratory studies.

The mathematical form of the model represented by Fig. 1 is consequently a generalization of the familiar equation for uptake and clearance, except that the uptake rate depends on two time-variable functions, the abundance of *Nitzschia* and the concentration of domoic acid in *Nitzschia* cells, and the uptake and clearance (depuration) rates are temperature dependent:

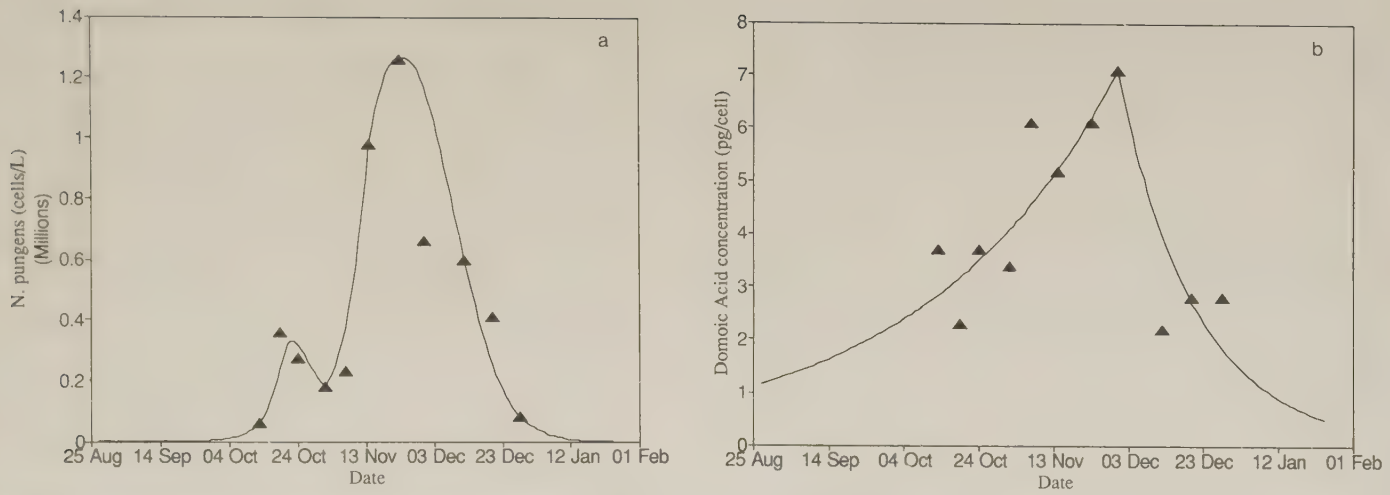

FIG. 2. Fitted curves representing 1988–89 field data from Bates et al. (1989). (a) *Nitzschia* concentration; (b) concentration of domoic acid in *Nitzschia* cells.

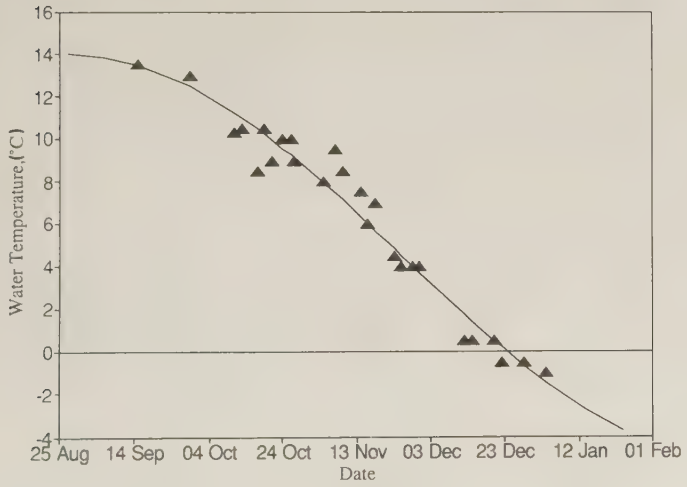

FIG. 3. Sinusoidal temperature distribution at 1 m in Cardigan River 1988 field data from Smith et al. (1990a).

$$dC_M/dt = F \cdot A \cdot C_A - D \cdot C_m$$

where F is the daily filtering rate of mussels, A is the concentration of *Nitzschia* in the water, C_A is the concentration of domoic acid in the algae, D is the daily depuration rate of domoic acid from the mussels, and C_M is the resultant concentration of domoic acid in mussel tissue. The variables A and C_A are represented by smoothed time series derived from field observations, while F and D are temperature-dependent quantities based on laboratory studies. Based on a review of earlier experimental studies (Winter 1978) as well as more recent experimental work by G. Wohlgeschaffen (pers. comm.), an F value of 40 L·mussel^{-1}·d^{-1} was used in the initial simulations (see Fig. 4a and 4d), and additional simulations were carried out with F set equal to 60 and 85 L·mussel^{-1}·d^{-1} as part of the sensitivity analysis of the model (see Fig. 4b and 4c).

Smoothed time series which drive the model are shown in Fig. 2 and 3. The fitted algal abundance with field data for the 1988–89 season (Bates et al. 1989) are displayed for comparison (Fig. 2a). Data on the concentration of domoic acid in the algal cells are fairly noisy (Fig. 2b) and the dramatic decline in domoic acid production at the peak of the bloom is poorly understood, so the data have been fitted simply by two exponentials. Temperature data for Cardigan Bay (Smith et al. 1990a) follow a sinusoidal curve (Fig. 3).

Four simulations are shown in Fig. 4. These runs differ in the filtering volumes and depuration rates assumed for *Mytilus*. Two sets of data are superimposed on the simulations; the solid squares represent data collected by Lorne Lea (Head, Department of Fisheries and Oceans Inspection Branch Laboratory, P.O. Box 1236, Charlottetown, P.E.I. C1A 7M8, pers. comm.), and the hollow squares show data reported by Bates et al. (1989). The experimentally determined filtering volume of 40 L·mussel^{-1}·d^{-1} was used for the simulations shown in Fig. 4a and 4d, while higher rates are used in the other two simulations. The depuration rate of 40%/d used for the simulations shown in Fig. 4a–4c is consistent with the observations of Novaczek (unpublished) that at low levels of accumulated domoic acid it takes approximately 2–3 d for mussels to depurate it.

The first simulation (Fig. 4a) generates reasonable levels of domoic acid in the mussels for most of the data points, but at the peak of the bloom where the levels approach 300 ppm the simulation underestimates these levels by a factor of approximately 2. Larger filtering volumes as shown in Fig. 4b and 4c improve the fit at the time of the bloom, but at the expense of worsening the fit for levels less than 100 ppm.

The last simulation (Fig. 4d) shows the effect of keeping the filtering volume at the experimentally determined value of 40 L·mussel^{-1}·d^{-1} but using a lower depuration rate of 20%/d. We obtain a good fit with the peak values, but the lower levels are seriously overestimated, especially in the period following the *Nitzschia* bloom.

Other simulations were carried out with different sets of parameters, but none of these resolved the difficulty of fitting both the lower and the peak values with the same simulation. These include simulations with different temperature dependencies, since experimental results are inconclusive and give values for Q_{10} in the range 0–5 (Winter 1978) (in the simulations shown in Fig. 4, both the grazing and depuration rates use a typical Q_{10} value of 2.2, although a range of other values were tried).

In the 1989–90 season the pattern was dramatically different. The peak cell concentrations were about 40% of those recorded

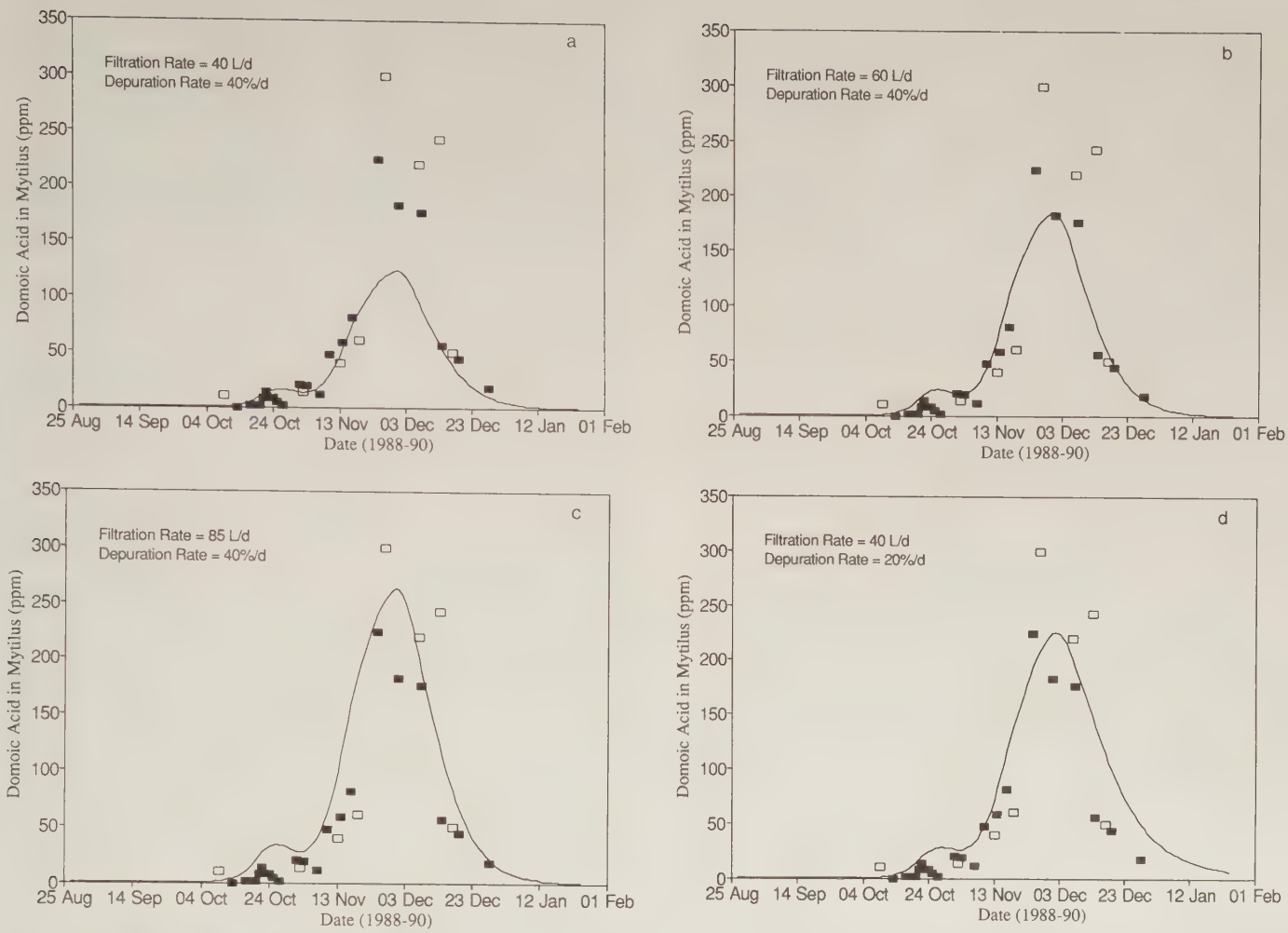

FIG. 4. Simulated domoic acid levels in mussels (solid line) compared with 1988–89 field data (squares). Solid squares are from L. Lea (pers. comm.); open squares are from Bates et al. (1989). (a) Filtering rate of 40 L·mussel^{-1}·d^{-1} and depuration rate of 40%/d; (b) Filtering rate of 60 L·mussel^{-1}·d^{-1} and depuration rate of 40%/d; (c) Filtering rate of 85 L·mussel^{-1}·d^{-1} and depuration rate of 40%/d; (d) Filtering rate of 40 L·mussel^{-1}·d^{-1} and depuration rate of 20%/d.

the previous year and the cellular domoic acid values were approximately the same, but the highest reported level of domoic acid in the *Mytilus* population was only about 15 ppm, far below the peak values of 200–300 ppm recorded at the end of 1988 (Smith et al. 1990b). Figure 5 shows the results of a simulation with a filtering rate of 40 L·mussel^{-1}·d^{-1} and a depuration rate of 40%/d, parameter values which gave the best fit to the lower points in the 1988 data but underestimated the peak values by a factor of 2; in 1989 the situation is reversed, and the same parameters instead overestimate the peak levels of domoic acid in *Mytilus* by a factor of 2. These later results suggest that ingestion and retention of domoic acid is very low when the concentrations are low, and this conjecture is supported by the data for September 1989, which indicate that there is virtually no domoic acid in mussels even though the domoic acid concentration in particulate material at that time averages about 0.1 μg/L (Smith et al. 1990b).

Discussion

A one-compartment uptake–clearance model with coefficients that depend only on temperature in a standard exponential fashion cannot account for the extremely high levels of domoic acid observed in Cardigan Bay *Mytilus* populations during the peak of the *Nitzschia* bloom in 1988, although the same model overestimates the peak levels of domoic acid found during the 1989 bloom. A simulation based on laboraory measurements of the filtering volume and depuration rate (Fig. 4a) gives very good results under nonbloom conditions but seriously underestimates the amount of domoic acid that accumulates in mussels at the height of the bloom. To reach the peak levels of the 1988 bloom, we must increase either the filtering volume or the depuration time by a factor of approximately 2 during the period of maximum *Nitzschia* abundance. In the absence of experimental evidence to support such changes, we are forced to speculate about processes that might account for the abnormally high levels of domoic acid found in *Mytilus*. It should be noted that the domoic acid levels reported for 1988 are much lower than those approaching 800 ppm found the preceding year (Bates et al. 1989), so we feel that the problem in fitting even the 1988 peak levels raises serious questions about our understanding of the dynamics of domoic acid in this system.

One obvious hypothesis is that the grazing rate increases in bloom conditions, which would correspond to a higher filtering

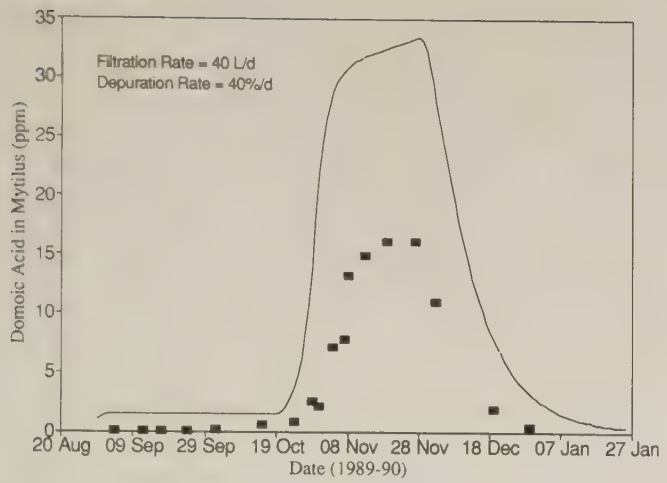

FIG. 5. Simulated domoic acid levels in mussels (solid line) compared with 1989–90 field data (squares). Solid squares are from Smith et al. (1990b). A filtering rate of 40 L·mussel^{-1}·d^{-1}; and a depuration rate of 40%/d was assumed.

volume. However, this is inconsistent with laboratory results that indicate an inverse relationship between filtering volume and *Nitzschia* concentration at the levels encountered in the field (G. Wohlgeschaffen, pers. comm.). Although these laboratory experiments were carried out with *Nitzschia* monocultures, the dominance of Cardigan Bay by *Nitzschia* during the period under investigation was so high that we feel it is unlikely that feeding rates in the field would follow a pattern completely at variance with the laboratory studies. These studies indicate that the ingestion rates at the peak of the 1988 bloom are close to the maximum that *M. edulis* can achieve, so the possibility that local concentrations of *N. pungens* higher than the reported values can lead to high accumulation rates does not offer a plausible explanation.

An alternative hypothesis is that the very high domoic acid content of the mussels results from retention of the neurotoxin, showing up as a drastic decrease in depuration rate under bloom conditions. As shown in Fig. 4d, a doubling of the depuration time fits the peak values well, although clearly the simulation does not reproduce the pre- and postbloom behaviour correctly, so this hypothesis must involve a change in depuration rate under bloom conditions rather than a constant rate lower than that measured in the laboratory.

There are several kinds of biochemical and physiological processes that might account for a decrease in depuration rate at the peak of bloom conditions. Under some circumstances, domoic acid might become chemically bound to some other substance in the mussel tissue or gut. Another possibility is a physiological response in the *M. edulis* that interferes with the depuration process, or possibly some microbial or other internal mehcanism that leads to production of domoic acid within the gut of the mussels. Another is a change in the utilization of *Nitzschia* at high concentrations, leading to its accumulation in organs other than those in the digestive system. This could be modelled by using a two-compartment model instead of the one-compartment one used here. It has also been suggested that differential temperature dependencies of uptake and depuration rates might play a role, although we have not been able to obtain quantitative information on possible patterns that could be used to test this hypothesis. It is difficult to see how temperature alone can account for the retention of domoic acid, since the depuration rate seems to return to normal values after the bloom.

At low levels of domoic acid availability the amount of domoic acid found in *Mytilus* is lower than predicted by the simulation model, as evidenced by the field data for September 1989 (Smith et al. 1990b). This may simply be due to preferential feeding on other sources, since *N. pungens* f. *multiseries* is not very abundant and is in fact outnumbered by a nontoxic form of *N. pungens*. However, during bloom conditions it is virtually the only available food source, so selective feeding cannot be a factor.

Conclusions

The results of these simulations indicate that the peak levels of domoic acid found in populations of *M. edulis* during *N. pungens* blooms can reach levels considerably higher than can be explained with a one-compartment uptake–clearance model of the domoic acid flux. The phenomenon seems to occur only when domoic acid concentrations are very high and manifests itself as a nonlinear relationship between the amount of domoic acid available in particulate form in the water column and the amount which accumulates in mussels. We have investigated two hypotheses to explain this. One, that grazing rates increase under bloom conditions, is at variance with laboratory data that show an inverse relationship between grazing rate and *Nitzschia* abundance. A more plausible hypothesis is that the time required for depuration of domoic acid from *M. edulis* populations during bloom conditions is considerably longer than the 2 or 3 d observed in laboratory studies (Novaczek, unpublished). Whether this is due to metabolic changes in *M. edulis* under conditions of high domoic acid stress or whether domoic acid is possibly retained in other tissues is not clear at this time. It might be that a two-compartment model of the dynamics of domoic acid through *Mytilus* is needed, but at present there is not sufficient empirical evidence to justify development of such a model.

As most of the shellfish toxin episodes (paralytic and diarrhetic) are usually associated with shellfish that feed on toxin-bearing dinoflagellates, and with accumulation and depuration of toxins that are species specific, our study suggests that such episodes may also be amenable for modelling, and consequently that theoretical analyses based on such models may prove a useful tool for testing the assumptions underlying the management of shellfish fisheries.

Acknowledgments

We thank several colleagues, particularly Drs. James E. Stewart, Ken H. Mann, and Donald C. Gordon of the Habitat Ecology Division, Dr. Robert Mohn of the Marine Fish Division, and Mr. Gary Wohlgeschaffen of Dalhousie University, for helpful discussions and criticism of the manuscript. We appreciate the keen interest and continued help of Mr. Lorne Lea at the Department of Fisheries and Oceans Inspection Branch Laboratory, Charlottetown, P.E.I.

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Mapping the Potential Spread of the Zebra Mussel (*Dreissena polymorpha*) in Ontario[1]

B. P. Neary

Ontario Ministry of Environment, Dorset Research Centre, Bellwood Acres Road, Dorset, Ont. P0A 1E0, Canada

and J. H. Leach

Ontario Ministry of Natural Resources, Lake Erie Fisheries Station, RR 2, Wheatley, Ont. N0P 2P0, Canada

Neary, B. P., and J. H. Leach. 1992. Mapping the potential spread of the zebra mussel (*Dreissena polymorpha*) in Ontario. Can. J. Fish. Aquat. Sci. 49: 406–415.

The European zebra mussel (*Dreissena polymorpha*) is now firmly established in the lower Great Lakes and in isolated locations in the upper Great Lakes. Dispersal is aided by high fecundity, a planktonic larval stage, and ability of the mussel to be transported via shipping and recreational boating. Almost all waterbodies in Ontario which are downstream of established colonies or which can be reached through boating are vulnerable to invasion. However, there are ecological requirements (derived from European literature) which limit successful zebra mussel reproduction. Using data from over 6000 inland lakes in the province, we have prepared maps which show the distribution of pH, calcium, and major access routes and which indicate our estimated potential for success or failure of zebra mussel colonization. The utility of the maps is dependent on the validity of the ecological requirements for successful development of zebra mussel larvae, which were developed in Europe. There is a need to test the chemical requirements of the zebra mussel in North American waters.

La moule zébrée (*Dreissena polymorpha*) originaire des eaux européennes a maintenant envahi le bassin inférieur des Grands Lacs et certains endroits isolés du bassin supérieur. Sa dispersion est facilitée par un taux de fécondité élevé, un stade larvaire planctonique et le trafic maritime. Presque toutes les nappes d'eau de l'Ontario situées en aval de bancs établis de moule zébrée ou accessibles par bateau sont vulnérables à l'invasion. Toutefois, des besoins écologiques relevés dans des publications européennes limitent le succès de la reproduction de cette espèce. À l'aide de données sur plus de 6 000 lacs intérieurs de l'Ontario, les auteurs ont préparé des cartes du pH, des teneurs en calcium et des principales routes d'accès; ces cartes donnent aussi un potentiel estimaté du succès ou de la faillite de la colonisation par la moule zébrée. L'aspect utilitaire de ces cartes dépendra de la validité des exigences écologiques du développement des larves de la moule zébrée, exigences établies dans les eaux européennes. Il est nécessaire de vérifier les besoins chimiques de la moule zébrée dans les eaux nord-américaines.

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The zebra mussel (*Dreissena polymorpha*), a small bivalve mollusc native to Europe, is now well established in the Great Lakes system. Initial colonization may have occurred in 1986, probably from larvae discharged in ballast water (Griffiths et al. 1991). The mussel was first observed in Lake St. Clair in June 1988 (Hebert et al. 1989) and in western Lake Erie in July 1988 (Leach 1991). By November 1990, the mussel had spread throughout Lake Erie and through the Welland Canal and Niagara River into the western basin and south shore of Lake Ontario. Isolated populations have been found in the other Great lakes as far west as Duluth, Minnesota, and as far east as the St. Lawrence River at Cornwall, Ontario. Rapid dispersal downstream is aided by a high fecundity and a pelagic larval stage. Upstream dispersion is mainly due to shipping and boating, with the zebra mussel carried either in ballast or attached to solid surfaces by byssal threads.

The zebra mussel is a serious pest in Europe (Clarke 1952) and is rapidly becoming one in North America. In high densities, it presents major problems for both civil engineering structures and for the ecology of infested waterbodies. One of the most harmful impacts is the colonization of intake cribs and pipes serving water treatment plants, power generating stations, and industries (Griffiths et al. 1989). Many installations on Lake Erie and Lake St. Clair have already been colonized to the point where flows have been restricted (Griffiths et al. 1989).

As an efficient filter feeder, the zebra mussel is capable of removing large amounts of seston from the water (Hebert et al. 1991; Kryger and Riisgård 1988). In western Lake Erie, mean Secchi disc transparencies for the May–November period almost doubled between 1988 and 1989 (Leach 1991). In some areas of Europe, the processing of seston by *D. polymorpha* is considered beneficial in water quality management (Reeders and Bij de Vaate 1990). However, there is concern in North America about the long-term effects of shifting so much organic matter from the pelagic to benthic zones. For example, mean chlorophyll *a* for the May–November period declined 54% in the western basin of Lake Erie between 1988 and 1990 (Leach 1991). Concentrations of chlorophyll *a* are now in the 1–2 $\mu g \cdot L^{-1}$ range which classes the basin at the oligotrophic end of the trophic scale (Leach 1991). Effects of these dramatic changes in water clarity and energy distrbution on invertebrate, aquatic plant, and fish communities have yet to be determined.

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Of further concern to fisheries managers is the rapid colonization of rocky reefs that are used for spawning by important commercial, sport, and forage species of fishes. Although reef substrates have been completely altered by the infestation of mussels, adverse impacts on walleye (*Stizostedion vitreum*) have not been observed to date (Fitzsimmons et al. 1991). There was initial concern that egg survival could be affected by high amounts of organic matter being deposited on the shoals as a result of mussel fecal and pseudofecal production. Potential impacts, if any, on fall spawners such as lake trout (*Salvelinus namaycush*) and lake whitefish (*Coregonus clupeaformis*) have yet to be assessed. Negative impacts of *D. polymorpha* on native unionids have been observed in Lake St. Clair and Lake Erie (Mackie 1990; Schloesser and Kovalak 1991). The rich diversity of the unionid community in these lakes may be reduced through extinctions of some species.

The Laurentian Great Lakes are by no means the only aquatic resource at risk in Ontario due to infestation by the zebra mussel. In addition to a share of four of the Great Lakes, Ontario is estimated to have approximately 262 000 lakes with a surface area greater than 1 ha (Cox 1978). The numbers and lengths of rivers and streams in the province have not been determined, so that extent of potential disruption of lotic ecosystems cannot be quantified. The potential for the spread of zebra mussels to these inland waters is of great concern to managers and municipal, industrial, and recreational users of these resources. Although North American experience to date has been limited to large lakes, European information (Mackie et al. 1989) indicates that smaller waterbodies on the scale of many of Ontario's inland lakes are vulnerable to impacts from the mussel.

Strayer (1991) has determined an area of potential invasion of the zebra mussel for North America based primarily on its thermal requirements as inferred from its distribution in Europe. However, there are chemical as well as thermal limits to the distribution of the zebra mussel. Sprung (1987) evaluated the chemical requirements for zebra mussel and determined that the larvae had very low survival rates at low pH and calcium concentrations.

Fortunately, the past decade has seen the development of extensive data bases of lake water chemistry, stimulated primarily by a need to assess the extent of lake acidification. Here, we use the Ontario Acid Sensitivity Data Base on over 6000 inland lakes in the province, along with maps of bedrock and surficial geology, and maps of major highways to construct maps of the relative probability of zebra mussel invasion to inland waters of the province. We also discuss problems of interpretation and extrapolation associated with current understanding of the chemical requirements of the zebra mussel. These preliminary maps may assist in focusing efforts to control the spread of the zebra mussel.

Methods

Water Chemistry Mapping

Sprung (1987) assessed the chemical requirements of zebra mussel reproduction. He reported no successful development of zebra mussel larvae at pH less than 7.4. Minimal survival of larvae was observed at calcium concentrations of 12 $mg \cdot L^{-1}$, and from data presented in Sprung's paper, 10% total rearing success of zebra mussel can be inferred to occur at approximately 20 $mg \cdot L^{-1}$. Based on these observations, critical water chemistry values were defined as pH of 7.4 and two calcium values: 12 and 20 $mg \cdot L^{-1}$. These calcium concentrations may prove to overestimate the area of the resource at risk due to zebra mussel invasion, since a recent review of the distribution of the mussel in European lakes showed that it was confined to lakes with a calcium concentration greater than 28 $mg \cdot L^{-1}$ (Ramcharan et al. 1991).

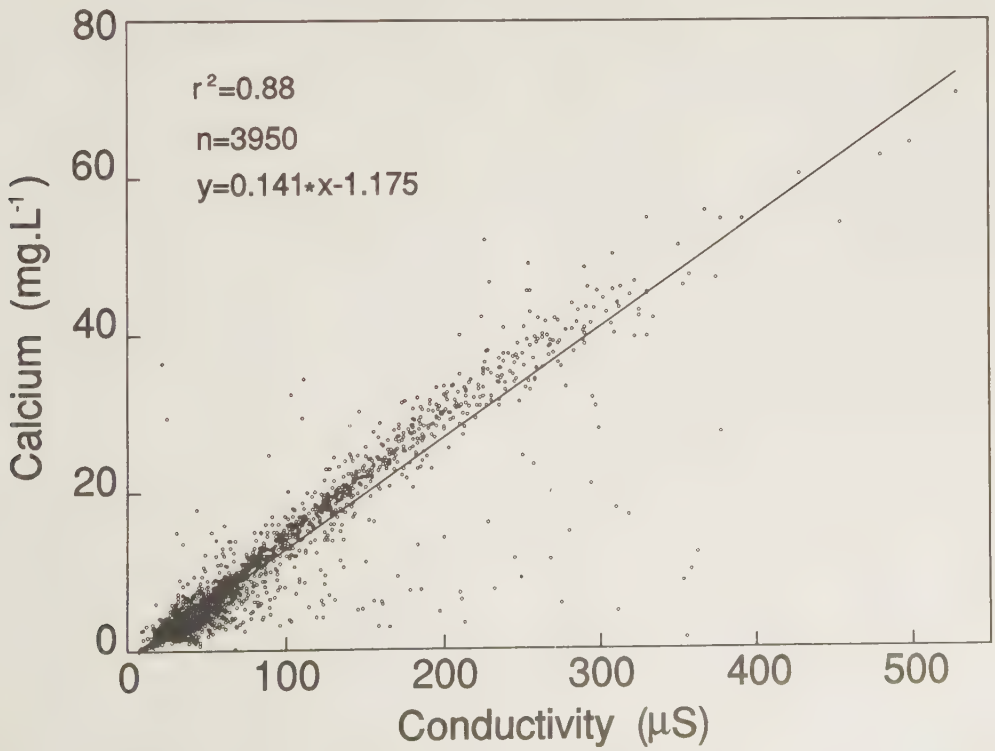

FIG. 1. Relationship between conductivity and calcium concentration in Ontario lakes.

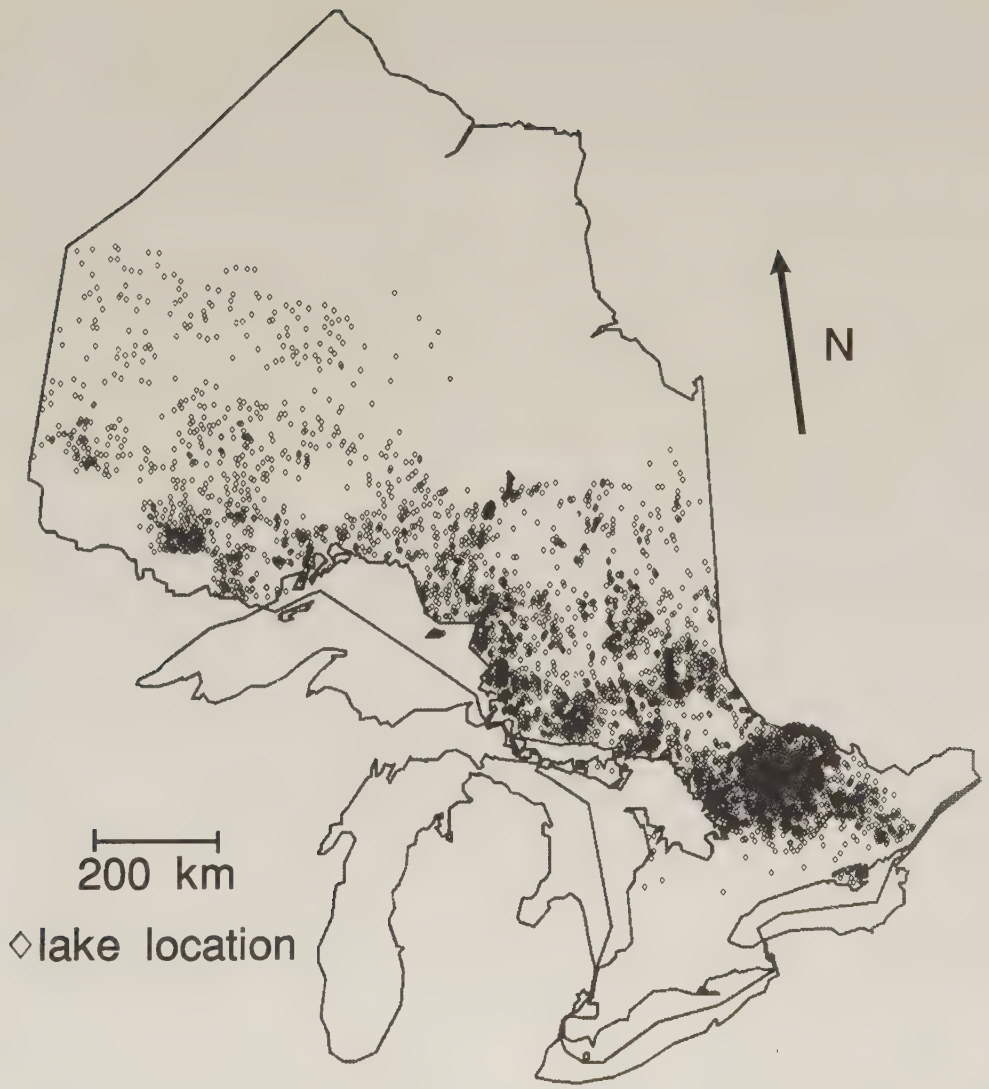

FIG. 2. Location of lakes in Ontario with measured pH and measured or calculated calcium.

Zebra mussel larval survival was qualitatively classified as "unlikely" if measured or mapped lake pH was less than 7.4. In areas of the province where mapped or inferred water chemistry had pH $\geq$ 7.4 and calcium concentrations between 12 and 20 mg·L^{-1}, survival likelihood was termed "possible." If measured or inferred pH was greater than 7.4, and measured or inferred calcium concentration was greater than 20 mg·L^{-1}, survival likelihood was classified "probable."

The water chemistry data base has been described in detail elsewhere (Neary et al. 1990). It is composed primarily of water chemistry data from a single sample from a midlake location, with winter being the most common sampling time. One of the requirements for the inclusion of data in the data base was that a measure of Gran alkalinity was available for the lake. There were considerable amounts of data also available on lakes that included fixed-endpoint alkalinity measurements, frequently with calcium data as well (Ontario Ministry of the Environment 1980, 1981, 1982, 1983). For the purposes of this mapping exercise, these lakes were also included. At higher alkalinity concentrations, the difference between fixed-endpoint and Gran alkalinity was felt to be insignificant.

A total of 3950 lakes had calcium measurements. Since calcium is the major cation in solution in Ontario lakes, it is highly correlated with conductivity (Fig. 1). This regression was used to estimate the calcium concentration on a further 2201 lakes with pH and conductivity data, but where calcium data were missing. This gave us a data base of 6151 lakes with pH measurements and either measured or estimated calcium concentrations. The location of these lakes is shown in Fig. 2. The lake water chemistry data were mapped using a SPANS geographic information system. The point data were converted to areas with a technique called potential mapping (TYDAC 1988). The assumptions inherent in potential mapping are that a point attribute (in this case lake pH or lake calcium) is related to the attribute of other, unsampled areas around it. The amount of interaction between points decreases with increasing distance between the points. In constructing pH and calcium water chemistry maps, each observation was given an inner radius of 500 m where neighbouring observations could not override the measured value. Each observation was then allowed an outer radius of 10 km. The value of the map for areas with no direct measurement was then calculated as the weighted sum of all points with influence on the map element.

There are large areas of the province where there were no observations (Fig. 2). In some areas (e.g. southwestern

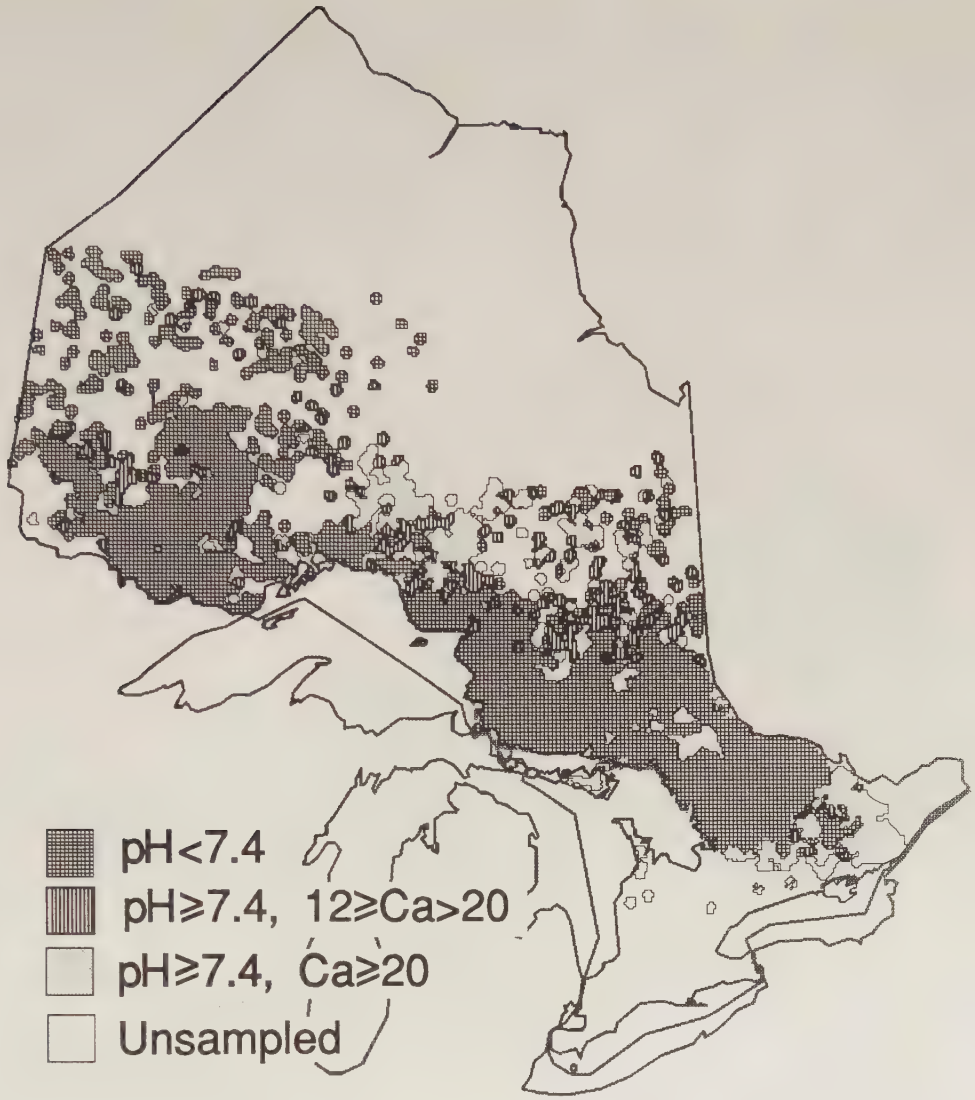

FIG. 3. Areas of Ontario with measured or calculated water chemistry of varying suitability for zebra mussel larvae survival.

Ontario), this is due to low densities of lakes. Other areas, particularly much of northern Ontario, are poorly sampled due to access difficulties. The lakes were sampled primarily because of interest in assessing the impact of acid deposition, and northern Ontairo receives much less acid deposition than the southern portions of the province. Lake acidification of softwater lakes in Ontario is directly correlated with the amount of acid deposition (Neary and Dillon 1988), and sampling effort was mostly directed to areas where acidification impacts were suspected.

Inferring Water Chemistry from Surficial and Bedrock Geology

The calcium concentration of lake water is primarily determined by the geology of the lake catchment. Lakes in areas with significant amounts of limestone or calcareous till will have higher calcium concentrations than those in areas dominated by granites or gneisses. Cowell (1986) produced maps of Ontario classifying the province into terrain types according to the capability of soils and rock in the area to neutralize acid deposition. This formed the basis for a national assessment of the potential of soils and bedrock to reduce acidity (Environment Canada 1988). Because the conditions which favour the survival of zebra mussels are the opposite of the conditions which render lakes susceptible to acidification, the use of the maps which predict the susceptibility of lakes to acidification was evaluated as an additional means of predicting surface water conditions which may be suitable for zebra mussel survival. Lakes were grouped according to the major terrain type of these maps, and the resulting classifiction was assessed as to its ability to predict pH and calcium concentration, based on measurements from the data base.

Evaluating Variability in the Chemical Data

One of the problems with mapping suitability based on one or more chemical criteria is the fact that water chemistry is variable both seasonally and annually. This is illustrated with a series of water chemistry data from Glen Lake, a small (16.3 ha) hardwater lake in central Ontario (45°08′N, 79°01′W). Glen Lake is not typical of lakes on the Precambrian Shield area of Ontario and owes its high calcium content and high pH to its situation on a localized dolomitic marble deposit.

TABLE 1. pH and calcium characteristics of Ontario lakes classified according to the potential of soils and bedrock in their drainage areas to reduce acidity (from Environment Canada 1988).

Geological class[a]	n	Median pH	% pH ≥ 7.4	Median Ca ($mg \cdot L^{-1}$)	% Ca ≥ 12 $mg \cdot L^{-1}$	% Ca ≥ 20 $mg \cdot L^{-1}$
Low	4557	6.54	15.0	3.54	13.3	6.0
Medium	1138	7.08	35.4	7.66	33.4	19.7
High	399	7.80	74.9	20.6	81.0	61.1
Organic	53	7.50	58.5	11.9	56.6	32.0

[a]Potential of soils and bedrock to reduce acidity.

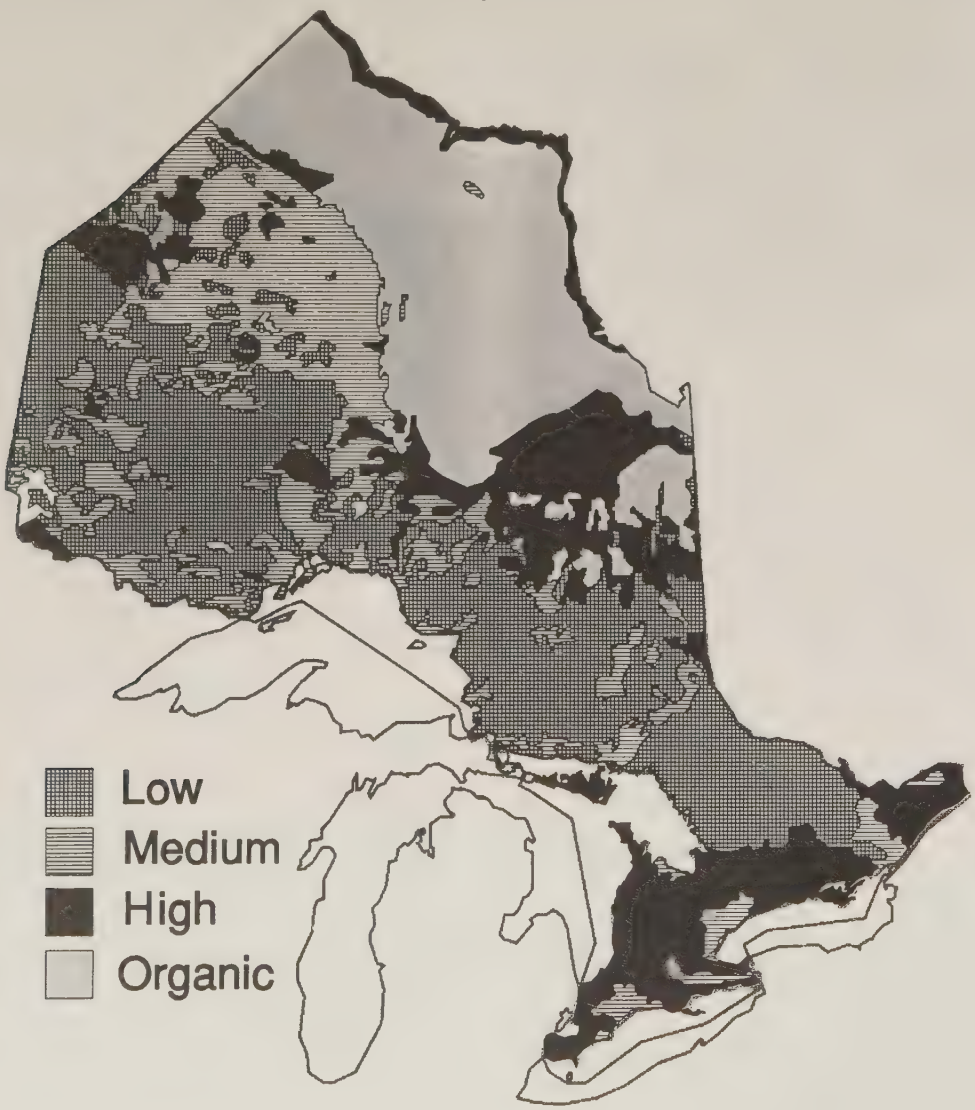

FIG. 4. Potential of soils and bedrock to neutralize acidity (from Environment Canada 1988).

Of a number of lakes monitored regularly for a number of programs by staff at the Dorset Research Centre, it is the highest in pH and calcium and represents a lake with conditions suitable for zebra mussel survival. This lake has been sampled bimonthly during the ice-free season each year since 1978 (Girard and Reid 1990), as well as twice annually under ice cover. The seasonal and long-term trends were plotted using seasonally detrended robust locally weighted regression methods developed by Bodo (1989).

The majority of data in the Ontario Acid Sensitivity Data base is from single grab samples either through the ice or from the epilimnion. The Glen Lake data are from sampling in the centre of the lake in the epilimnion during stratification, or a volume-weighted water column sample during the period when the lake is not stratified.

Assessing Accessibility

In addition to suitable water conditions, ease of access will likely influence the probability of zebra mussel invasion. Primary vectors for dispersal of zebra mussel in inland waters are thought to be boats and bait buckets. The inland waters of Ontario at highest risk are those with direct boat access to the Great Lakes. In Europe, canals have enhanced the dispersal of the mussel (see Mackie et al. 1989 for a review of European dispersal mechanisms). Two canal systems (the Trent–Severn and the Rideau) are undoubtedly at highest risk of invasion. In

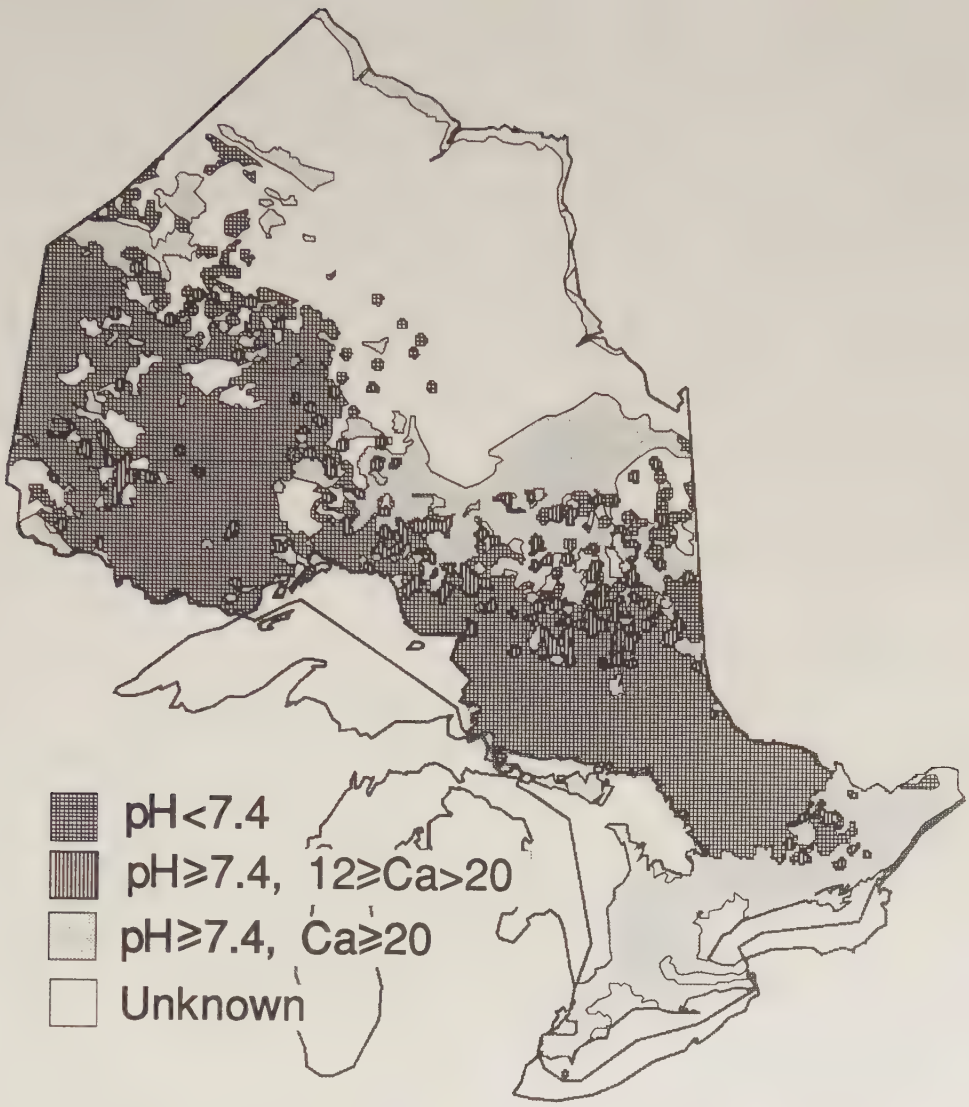

FIG. 5. Combination of areas with measured or calculated water chemistry (Fig. 3) with areas of low and high potential to reduce acidity (Fig. 4) to produce conditions of varying suitability for zebra mussel larvae survival.

November 1990, zebra mussels were found on the hull of a boat in the Rideau Canal near Ottawa, Ontario. Boats which are transported over land from infested waters form another potential dispersal vector because zebra mussel larvae can potentially survive in boat livewells or bilges, and adults can survive out of water for several days depending on temperature and humidity. Boat-related dispersal vectors require road access to lakes. A further classification of likelihood of zebra mussel invasion ("very probable") was assigned to areas where water chemistry requirements were met and access (defined as being within 10 km of a major highway) was good.

Results

Maps of Water Chemistry

Maps of calcium with three classifications ($Ca < 12\ mg \cdot L^{-1}$, $12 \geq Ca < 20\ mg \cdot L^{-1}$, and $Ca \geq 20\ mg \cdot L^{-1}$) were produced for Ontario. Maps with two pH classifications were then imposed on the calcium maps to produce a map with three water chemistry classifications: (a) $pH < 7.4$, (b) $pH \geq 7.4$ and $Ca \geq 12\ mg \cdot L^{-1}$, and (c) $pH \geq 7.4$ and $Ca \geq 20\ mg \cdot L^{-1}$ (Fig. 3). Generally, the map of areas of high pH aligned almost exactly with areas of high calcium. The only exception was a relatively small area in the vicinity of Sudbury, Ontario. This area has received large amounts of sulphur loading from smelters operating in the area and had lakes with high calcium concentrations coupled with relatively low pH. The different classes on Fig. 3 represent areas where measured water chemistry in inland lakes is suitable ($pH \geq 7.4$ and $Ca \geq 12\ mg \cdot L^{-1}$) or unsuitable ($pH < 7.4$ or $Ca < 12\ mg \cdot L^{-1}$) for zebra mussel larvae survival, based on survival criteria inferred from Sprung's (1987) assessment.

Inferring Water Chemistry from Surficial and Bedrock Geology

Grouping lake calcium and pH by the surficial and bedrock geological types yielded two useful classifications (Table 1). Grouping was done on digitized versions of the 1:1000000 maps published by Cowell (1986). The resolution of these maps cannot be adequately reproduced at the scale of a journal page, so

the lower resolution (1:7500000) surficial and bedrock geology maps published by Environment Canada (1988) are shown here (Fig. 4). Areas classified as having a low potential to reduce acid deposition had 15% of lakes with pH $\geq$ 7.4 and 13.3% of lakes with measured or estimated calcium concentrations $\geq$12 mg·L^{-1}. Areas with a high potential to reduce acid deposition had 75% of lakes with pH $\geq$ 7.4 and 81% of lakes with measured or estimated calcium $\geq$12 mg·L^{-1}. Lakes in the other two geological classifications had pH and calcium distributions which bracketed the critical pH and calcium values and had error terms which made them unacceptable for classification.

The map of water chemistry (Fig. 3) was superimposed on the two geology classes, and the result is shown as Fig. 5. For this map, the measured water chemistry was given priority over the geological theme, and only the two classes considered to be useful for predicting water chemistry were used. Areas of the province where geology was inappropriate for predicting water chemistry (the "medium" and "organic" areas from Fig. 4) were not assigned.

Seasonal and Annual Variability of pH and Calcium

The seasonal and long-term trends in the data series of pH and calcium from Glen Lake are shown in Fig. 6. The strong seasonal variability of both parameters is evident. Seasonal fluctuations contributed 78.5 and 24.5% of total variability in pH and calcium, respectively.

Zebra Mussel Invasion Probability Map

Maps of good road access were constructed by defining a corridor extending 10 km to either side of major highways of the province (Fig. 7). The water chemistry map as extended by geological classification (Fig. 5) was imposed on this access map, and areas where water chemistry was measured or predicted to be suitable for zebra mussel and road access was good were defined as "very probable" to be invaded by zebra mussels (Fig. 8). Also shown on Fig. 8 are two temperature isotherms, determined by Strayer (1991) to represent potential northern limits for the invasion of zebra mussels.

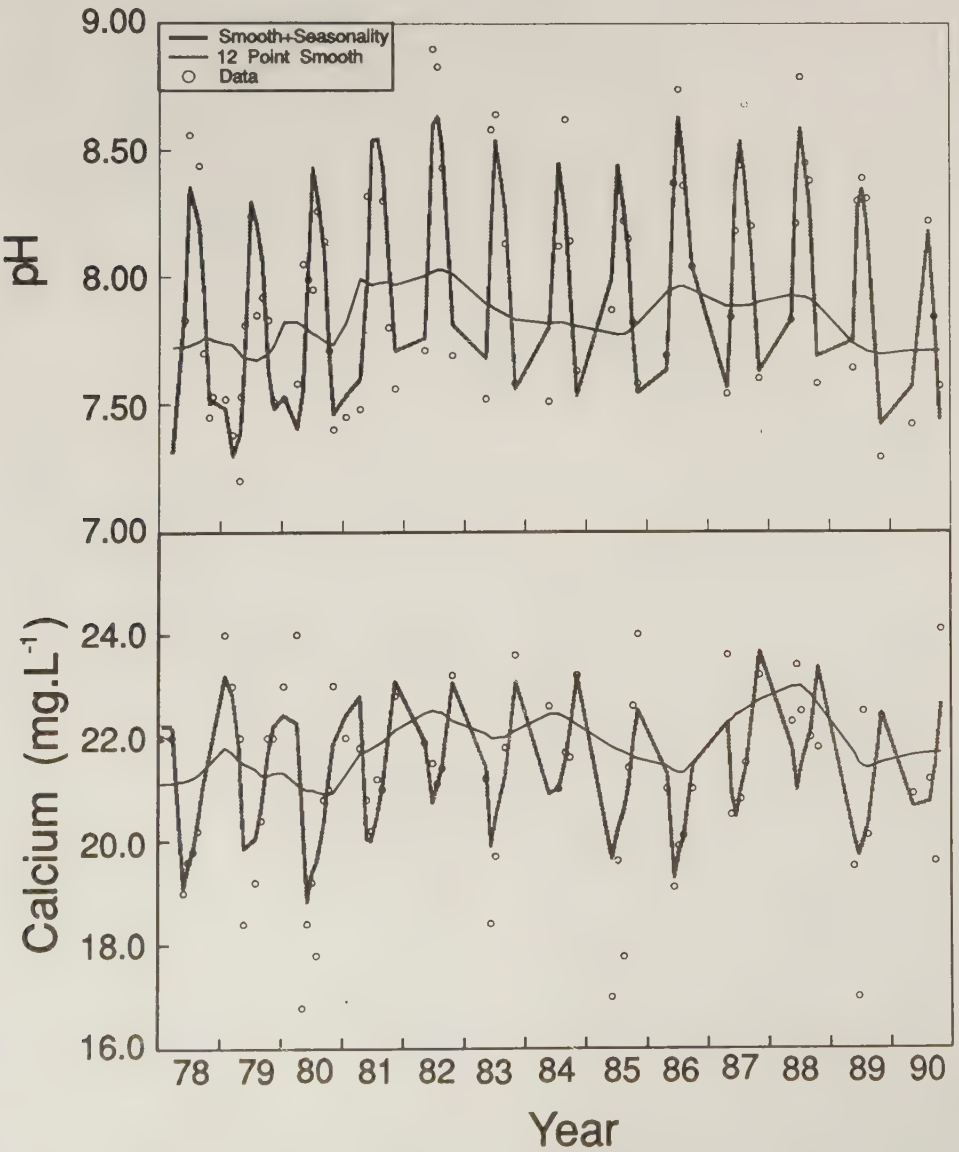

FIG. 6. Annual and seasonal variability of pH and calcium in Glen Lake, Ontario, 1978–90.

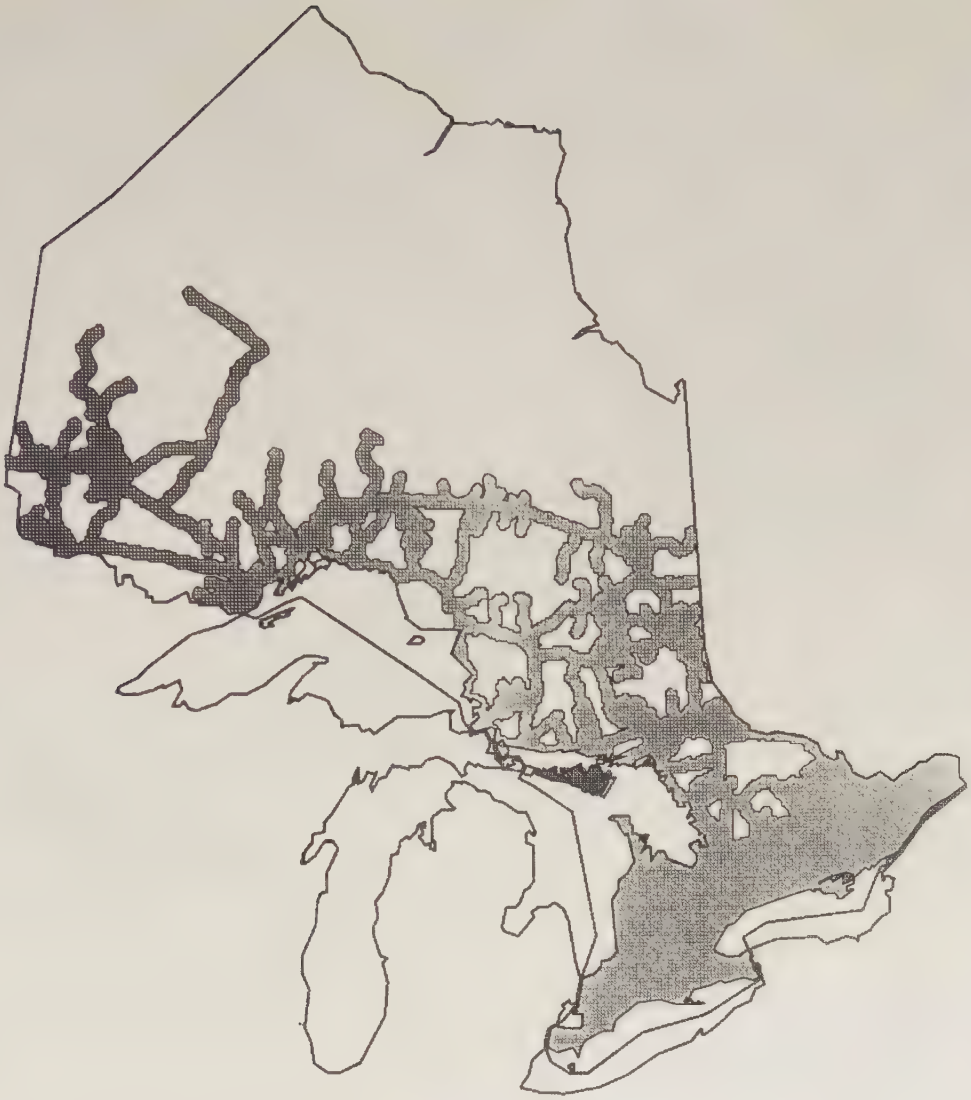

FIG. 7. Map of areas with good road access from major highways in Ontario.

Discussion

The large data base of water chemistry accumulated as part of the Acid Precipitation in Ontario Study proved extremely useful in classifying areas of known water chemistry according to published water chemistry requirements for successful reproduction of the zebra mussel. Classifying lakes according to a combination of bedrock and surficial geology considerably extended the area of the province which could be mapped as having conditions likely to be suitable or unsuitable for zebra mussel invasion.

The use of corridors along major highways to assign a higher probability of zebra mussel infestation probably has more validity in sparsely populated areas of northern Ontario than in southern Ontario. The southern portion of the province has an excellent network of secondary roads which can provide good access to lakes and rivers and which is not captured by the map. In northern Ontario, access in areas not close to major highways is considerably more limited, although some areas are accessible through secondary roads or logging roads. Fly-in fishing operations represent another potential vector for dispersal of zebra mussels which is not evaluated here.

The utility of these maps is strongly contingent on the validity of the chemical requirements for successful zebra mussel larval development which, to date, have been examined only in Europe. Sprung (1987) did not examine the survival of larvae at calcium levels between 12 and 0 $mg \cdot L^{-1}$, which is the range of calcium concentrations for the majority of Ontario lakes. The pH limitation of 7.4 is also problematic, since both pH and calcium concentrations are not static in a lake environment. Further, the pH limit of 7.4 was derived from a study where the zebra mussel was given no opportunity to acclimate to lower pH or calcium conditions.

Most of the data in the lake surveys represent subsurface "grab" samples. The seasonal and annual data from Glen Lake cover the periods of the year when grab samples were taken. Clearly, pH and calcium undergo significant seasonal variations in lakes. Variations in the littoral area, where zebra mussel colonization would be most probable, could be significantly greater. pH, in particular, would fluctuate in the inshore areas, with decreases occurring in the spring associated with snowmelt and runoff from adjacent shorelands and increases occurring later in the summer associated with respiration. Glen Lake may not be typical of larger lakes, since it receives a large portion of its input from runoff from its local watershed. Biol-

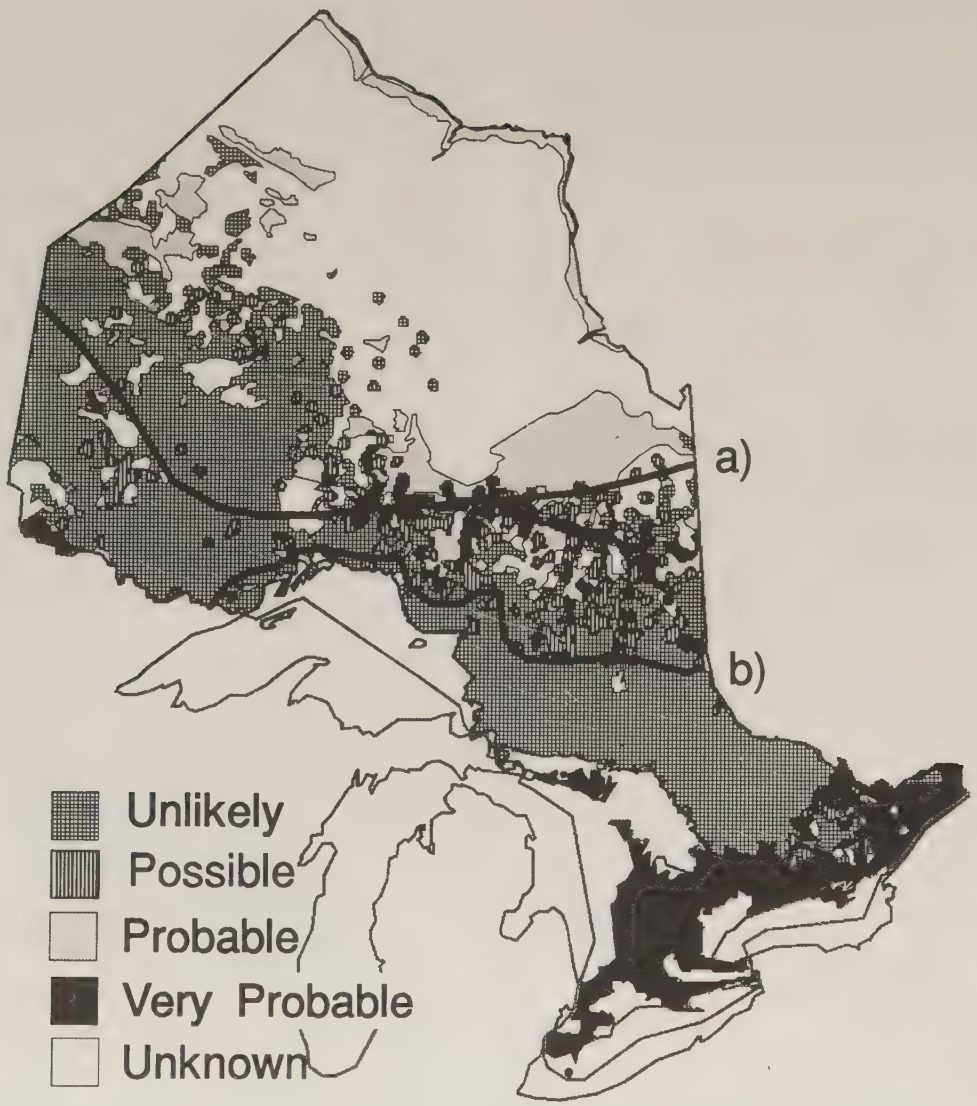

FIG. 8. Map of Ontario showing the relative probability of zebra mussel invasion. Isotherms for (a) mean annual temperature of 0°C and (b) mean January temperature of −15°C are shown. These temperatures may represent the northern extent of the range of the zebra mussel (Strayer 1991).

ogists attempting to further define the chemical requirements of the zebra mussel should bear in mind the variable nature of lake water chemistry. Limnologists attempting to classify lakes according to these or other water chemistry parameters should also attempt to address this type of variability.

The ecological problems associated with zebra mussel infestation are not a function of its mere presence, but are related to its abundance. Factors which will probably control its density (suitability of substrate, productivity, area of littoral zone) were not evaluated here. These data are not typically available for many of the lakes in Ontario.

The map presented here shows that there is a wide range of probability of invasion of the zebra mussel to inland lakes in Ontario. The likeliest systems to be invaded by the zebra mussel are lakes and rivers along two major waterway systems. The lakes along these systems have excellent access both by road and by direct passage of boats from Lake Ontario and Georgian Bay on Lake Huron. They are characterized by high calcium concentrations and are meso- to eutrophic, providing an excellent food base for the mussel.

Acknowledgements

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Establishment of Gobiidae in the Great Lakes Basin

David J. Jude

Center for Great Lakes and Aquatic Sciences, The University of Michigan, Ann Arbor, MI 48109-2099, USA

Robert H. Reider

Technical and Engineering Services, The Detroit Edison Company, Detroit, MI 48226, USA

and Gerald R. Smith

Museum of Zoology, Division of Fishes, The University of Michigan, Ann Arbor, MI 48109-1079, USA

Jude, D. J., R. H. Reider, and G. R. Smith. 1992. Establishment of Gobiidae in the Great Lakes basin. Can. J. Fish. Aquat. Sci. 49: 416–421.

A tubenose goby (*Proterorhinus marmoratus*), a European endangered species native to the Black and Caspian seas, was recovered on 11 April 1990 from the travelling screens of the Belle River Power Plant located on the St. Clair River, Michigan. Subsequently, anglers caught three round gobies (*Neogobius melanostomus*) in the St. Clair River near Sarnia, Ontario. Thirty-one tubenose gobies and 11 round gobies were impinged or trawled at or near the Power Plant in the fall and winter of 1990–91. Nine round gobies (29–61 mm total length) are believed to be young-of-the-year. These species were probably transported to the Great Lakes in ballast water, may have successfully colonized the St. Clair River, and will probably spread throughout the Great Lakes. They are expected to impact directly other benthic fishes, such as sculpins (*Cottus* spp.), darters (*Etheostoma* spp.), and logperch (*Percina caprodes*), and in turn act as prey for walleye (*Stizostedion vitreum*).

Un gobie à trompe (*Proterorhinus marmoratus*), un espèce en danger de disparition indigène aux mers Noire et Caspienne, a été trouvé le 11 avril 1990 dans les tamis mobiles de la centrale électrique Belle River située sur les rives de la rivière Sainte-Claire, au Michigan. Par la suite, des pêcheurs sportifs ont capturé trois gobies à taches noires (*Neogobius melanostomus*) dans la même rivière, près de Sarnia (Ontario). Au cours de l'automne et de l'hiver de 1990–1991, 31 gobies à trompe et 11 gobies à taches noires ont été trouvés dans les tamis mobiles de la centrale électrique ou capturés au chalut dans les environs. Neuf des gobies à taches noires mesurant de 29 à 61 mm de longueur totale avaient moins d'un an. La présence de ces deux espèces dans les Grands Lacs peut être attribuée au déversement des eaux des ballasts d'un bateau étranger; du point de déversement, elles ont colonisé la rivière Sainte-Clair et envahiront probablement tout le bassin des Grands Lacs. On prévoit qu'elles auront un impact direct sur d'autres espèces de poissons benthiques, dont les chabots (*Cottus* spp.), les dards (*Etheostoma* spp.) et le fouille-roche (*Percina caprodes*), et qu'elles seront la proie du doré jaune (*Stizostedion vitreum*).

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We report herein the establishment of another two exotic fish species into the Great Lakes basin, probably via ballast water discharge from a foreign tanker which took on water somewhere in the Black Sea. Two species of gobies, the round goby (*Neogobius melanostomus*) (Fig. 1a) and the tubenose goby (*Proterorhinus marmoratus*) (Fig. 1b), were collected in the St. Clair River by three methods: impingement on a power plant's cooling water intake travelling screens, angling, and bottom trawling. The occurrence of the round goby was also reported by Crossman et al. (1991).

Gobies are benthic fish, with characteristic fused pelvic fins forming a suctorial disk, which is used for anchoring the fish in fast-flowing water. They are nest guarders and eat a variety of benthic food organisms, including bivalve mollusks, so they could become another predator on the recently, and possibly concurrently, introduced zebra mussel (*Dreissena polymorpha*) (Hebert et al. 1989). In general, gobies are robust, known for their ability to adapt to low salinities and to habitats not accessible to other fishes. Some species, however, are sensitive to environmental change.

The family Gobiidae has a worldwide distribution, occurring in both fresh and saltwater. Sixty-eight species are native to the Atlantic and Pacific coasts of North America; 10 enter freshwater (Robins et al. 1991).

The St. Clair River is an active navigation channel, linking the oceans of the world with the Great Lakes. The river is a 63-km-long strait connecting Lake Huron to Lake St. Clair. Midchannel depths range from 8.2 to 21.5 m and current velocity can approach 1.8 m/s (Derecki 1984). It is periodically dredged and has a mean annual discharge rate for the period 1900–89 of 5182 m^3/s (USACOE 1990). The monthly averages of chloride levels at Marine City, Michigan, ranged from 6.8 to 11.0 mg/L for the March–December 1990 period.

Methods

Fish were collected using three different methods. Some specimens were collected off the travelling screens (0.95-cm bar mesh) of the intake structure of the Detroit Edison Company's (DECo) Belle River Power Plant (Fig. 2). Sampling of

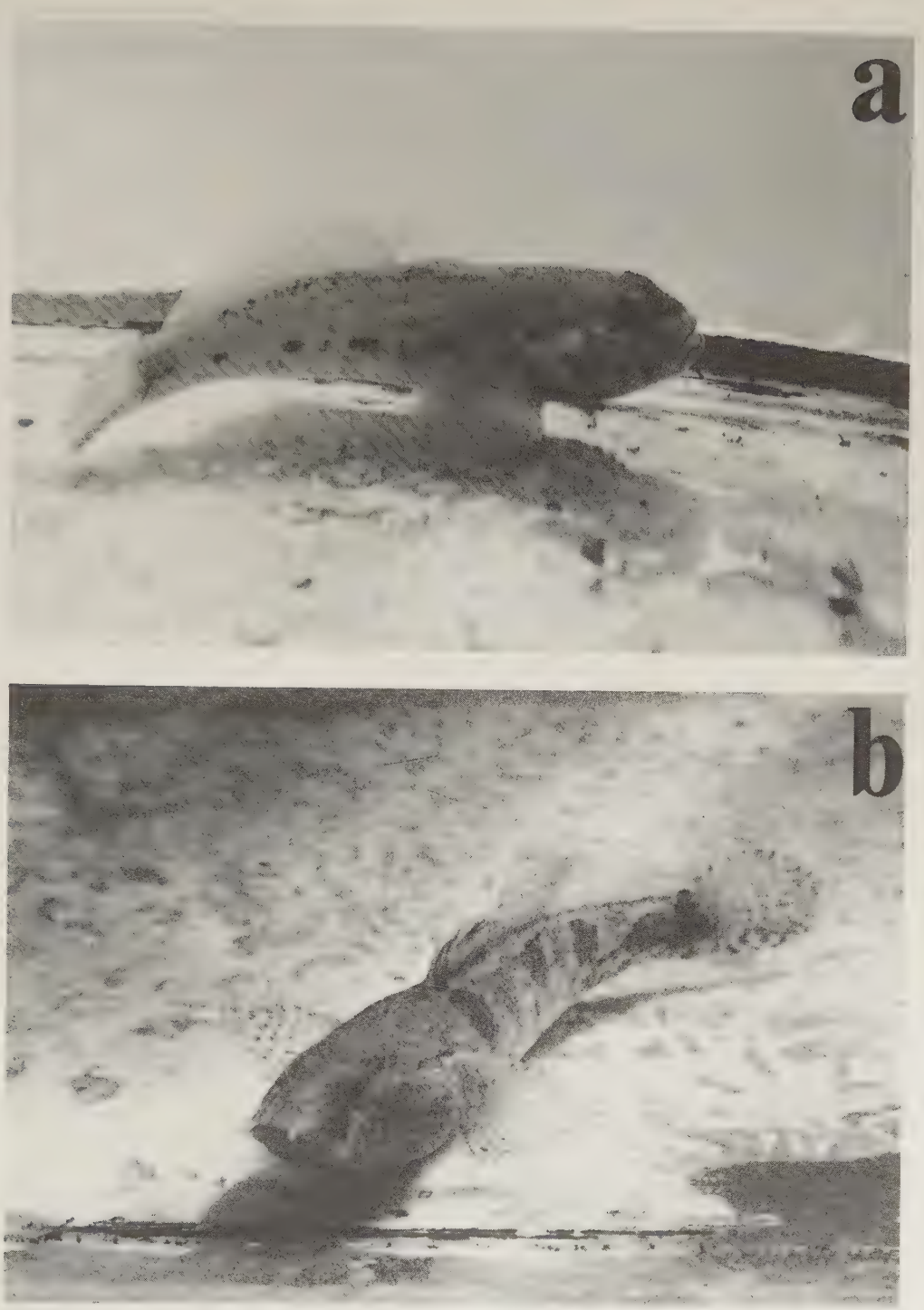

FIG. 1. (a) Round goby (*Neogobius melanostomus*) 118 mm total length and (b) tubenose goby (*Proterorhinus marmoratus*) 83 mm total length collected from the St. Clair River near St. Clair, Michigan.

the screens was part of a 1-yr study that commenced in March 1990. Sampling was conducted once a week for a 24-h period. The Belle River Power Plant is located on the west side of the St. Clair River approximately 6.5 km south of St. Clair, Michigan. Cooling water flow ranged from 492 to 1741 m^3/min during the course of the 1-yr study. All fish were measured (total length to the nearest millimetre), while only those that died were weighed (to the nearest 0.1 g). Most fish were transported to the University of Michigan Museum of Zoology, Division of Fishes, where they were identified and cataloged.

Three round gobies were obtained on 28 June, 18 July, and 23 September 1990 from three anglers fishing in the St. Clair River near Sarnia, Ontario, which is approximately 26 km north of the Belle River Power Plant.

Thirty-four specimens of both species were trawled on three dates, 30 November and 12 and 17 December 1990, from a small cove south of the Belle River Power Plant by DECo and Michigan Department of Natural Resources (MDNR) personnel using 4.9-m, semi-balloon, nylon bottom trawl having a cod end interliner of 1.3-cm stretch mesh. Most gobies were collected in depths of 1.5–4.6 m; sizeable beds of submerged

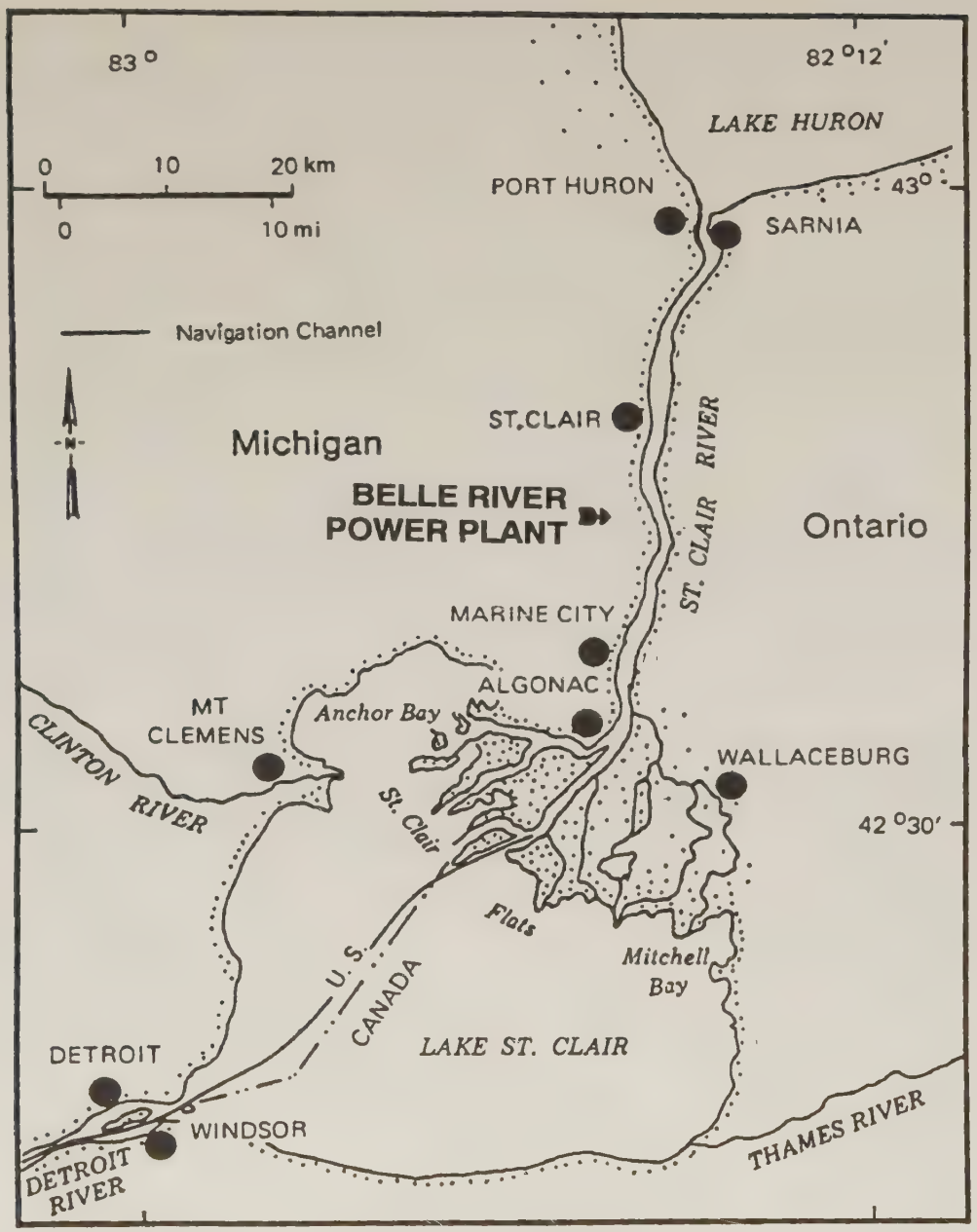

FIG. 2. The St. Clair River, connecting channel between Lake Huron and Lake St. Clair, showing the location of the Belle River Power Plant. The trawling sites were just south of the plant. Also shown is Sarnia, Ontario, where fishermen caught at least three specimens. Adapted from Edsall et al. (1988).

aquatic macrophytes (*Elodea canadensis, Myriophyllum* spp., and *Potamogeton* spp.) and the alga *Chara* sp. were observed in this cove.

Results and Discussion

Thirty-one tubenose gobies and 14 round gobies have been captured by a variety of methods and at different times starting in spring 1990 in the St. Clair River (Fig. 2). Four tubenose gobies were impinged at the Belle River Plant on 11 April and 30 November 1990 and 7 February 1991, and 27 were trawled from a cove adjacent to the plant's intake structure on 30 November (one) and 12 (three) and 17 December (23). Water temperatures on the six dates were respectively 4.0, 7.9, 1.7, 8.0, 6.0, and 6.6°C. As large quantities of submerged aquatic macrophytes were collected in the trawls that contained all but one of the trawled gobies, it was apparent that the fish were associated with this type of habitat in late fall. Nearshore spring and fall movements by the gobies are suggested by these catch data, particularly the impingement data, as the gobies only appeared on the travelling screens during these periods and February 1991. The travelling screens are located in 6 m of water and form the west bank of the river in this area. Trawling that was conducted in areas that were deeper, had a faster current, and were devoid of aquatic macrophytes caught only one tubenose goby. Measurements of length and weight were obtained on the four impinged tubenose gobies and 20 of the trawled gobies. The 24 specimens ranged in length from 45 to 87 mm (Fig. 3).

The fish collected in trawls were kept alive and placed in aquaria for observation of their behavior. Both goby species remained on the bottom, closely associated with rocks. Tube-

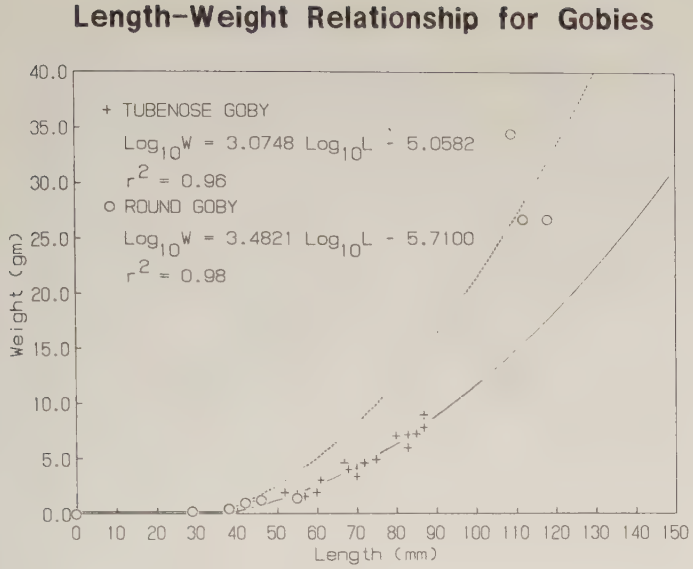

FIG. 3. Length–weight data for tubenose (*Proterorhinus marmoratus*) and round gobies (*Neogobius melanostomus*) impinged and caught with trawls and by hook and line from the St. Clair River during 1990–91.

nose gobies appeared to be more cryptic than the other benthic fish species, round goby, mottled sculpin (*Cottus bairdi*), logperch (*Percina caprodes*), and rainbow darter (*Etheostoma caeruleum*), with which they shared the aquarium. Tubenose gobies were not nearly as aggressive as these species at attacking food items introduced into the aquarium.

Tubenose gobies are native to fresh and slightly brackish waters of the Black and Caspian seas (Fig. 4). Lelek (1989) stated that the tubenose goby was originally a rheophilic species that has adapted itself to lentic habitats, particularly canals and ponds overgrown with submerged vegetation. Their distribution around the inshore areas of the Black and Caspian seas indicates their potential for widespread occupation of inshore habitats with cover, especially plants, in the lower Great Lakes. Tubenose gobies reach about 5 yr of age at a size of about 100–110 mm, suggesting that most of the fish we collected were adult fish. Tubenose gobies first spawn as yearlings or age 2 in April and May; most die after spawning. Males guard the nest, a shell, or shallow depression in shallow water. This fish is listed as an endangered species in Russia because of alterations to its habitat.

Tubenose gobies may be identified by the following characters: two dorsal fins, pelvic fins joined on the thorax, 45–48 scales in the lateral series (Miller 1986), no lateral line on the body, anterior nostrils extend in tubes over the upper lip, the first gill slit partly occluded, and four gill rakers on the anterior edge of the first arch.

The 14 round gobies were collected from the Belle River Power plant travelling screens (four), from trawling in the cove adjacent to the plant's intake structure (seven), and from three anglers fishing near Sarnia, Ontario (three). The 14 round gobies ranged from 29 to 118 mm (Fig. 3) and were first collected by a fisherman using worms on 28 June. The round goby is an abundant species in the Black and Caspian seas (Fig. 5) and, despite its relatively small size, is harvested commercially there (Moyle and Cech 1988). Round gobies reach about 80–110 mm standard length (females) to 100–130 mm standard length (males) (Berg 1949) in their first year of life, strongly suggesting that the smaller specimens we collected (<65 mm, 9 of the 14 collected) were young-of-the-year (YOY), while the other five were probably age-group 1. The YOY fish are clear evidence of natural reproduction. The YOY are not likely derived from ballast water discharges, in view of (1) the fine-mesh screens covering ship intakes, which should restrict all but larval fish and egg introductions, and (2) relatively high abundance of round gobies in many of the collections. However, Carlton (1985) has noted that corrosion of screens leaving holes up to 3 cm on some ships may allow larger fish to pass with ballast water discharges. Round gobies can reach lengths of 215–250 mm and an age of 5 yr (Berg 1949).

The round goby occurs in coarse gravel, shelly, and sandy inshore areas in the Black and Caspian seas; they migrate to deeper water (50–60 m) in winter (Miller 1986). They also occur in the middle and lower reaches of rivers in fresh to slightly brackish water. They feed chiefly on bivalves, amphipod crustaceans, and polychaetes, but chironomids, small fish, and fish eggs are also found in their diet. Consumption of fish suggests that round gobies might have a detrimental impact on native species through competition for food and predation on eggs and young fish. Since the round goby may grow to sizes of 215–250 mm, which is a much larger adult size than the darters (*Etheostoma* spp.), sculpins (*Cottus* spp.), and logperch, with which they currently share habitat in the St. Clair River, round gobies may consume the YOY and yearlings of these species. The round goby spawns over a prolonged period, April–September in some areas, but the spawning season is less protracted (May–June) in others. This species is a repeat spawner (up to six times) every 18–20 d. They deposit large (3.9 × 2.2 mm) eggs under or between stones; males guard the nest vigorously. Fecundity is 328–5221 per female. There is sexual dimorphism in age and size at maturity, with males generally sexually mature at 3–4 yr (age 2–3); females mature earlier (age 1–2). Males die after spawning; they do not eat, become emaciated, and some show skin ulceration (Miller 1984a). Members of the genus *Neogobius* reach a relatively large size among goby species and their young do not pass through a planktonic stage (Miller 1984b). Round gobies compose most of the gobiid commercial catches from the Sea of Azov, Romanian, and Bulgarian waters.

Specimens can be identified by the following characters: two dorsal fins, pelvic fins joined on the thorax, 49–55 scales in the lateral series, no lateral line on the body, a prominent black blotch in the posterior part of the first dorsal fin and on the anteriodorsal corners of the pectoral fin (Miller 1986), and about 12 gill rakers on the anterior edge of the first arch.

Individuals in our aquaria fed aggressively on dragonfly naiads and waxworms. They are a benthic species, resting on rocks with their large pectoral fins. The larger ones preferred to be in a crevice or under rock cover. The largest one was once scared and buried his whole body in the pea gravel on the bottom of the aquarium in an apparent predator avoidance reaction. The St. Clair River with its fast current, hard clay, and sometimes rocky bottom, and diverse benthic fauna is probably optimal habitat for this species.

The most probable route of entry for both species of gobies into the Great Lakes basin was transport by a freighter(s) from the Black Sea in Europe. Ballast water discharges have been responsible for a large number of exotic introductions (see Carlton 1985; Moyle 1991), probably including the zebra mussel. Gobies have successfully colonized many other habitats worldwide. Two Japanese goby species have been established in San Francisco Bay, California, via introductions (Moyle and

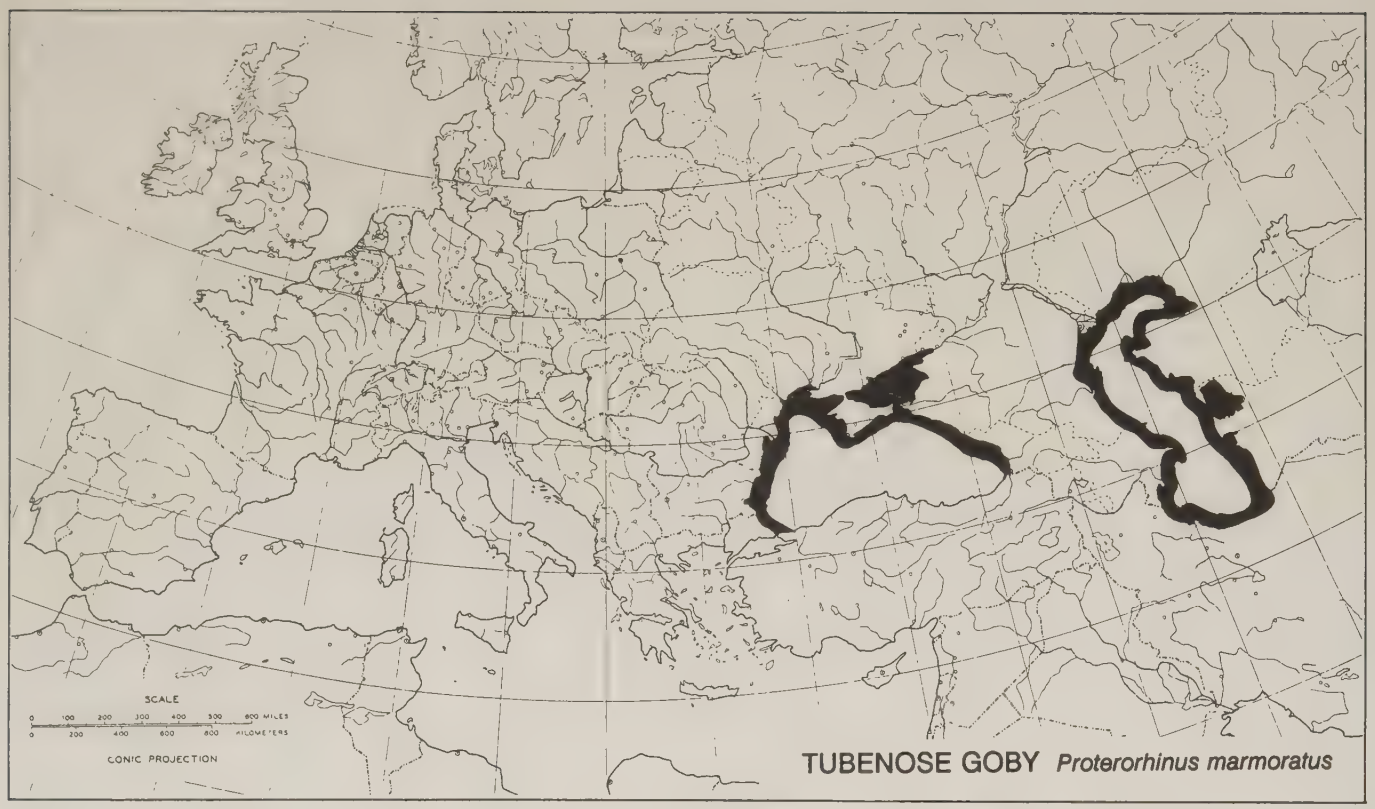
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FIG. 4. Worldwide distribution map for the tubenose goby (*Proterorhinus marmoratus*). Circles indicate major cities.

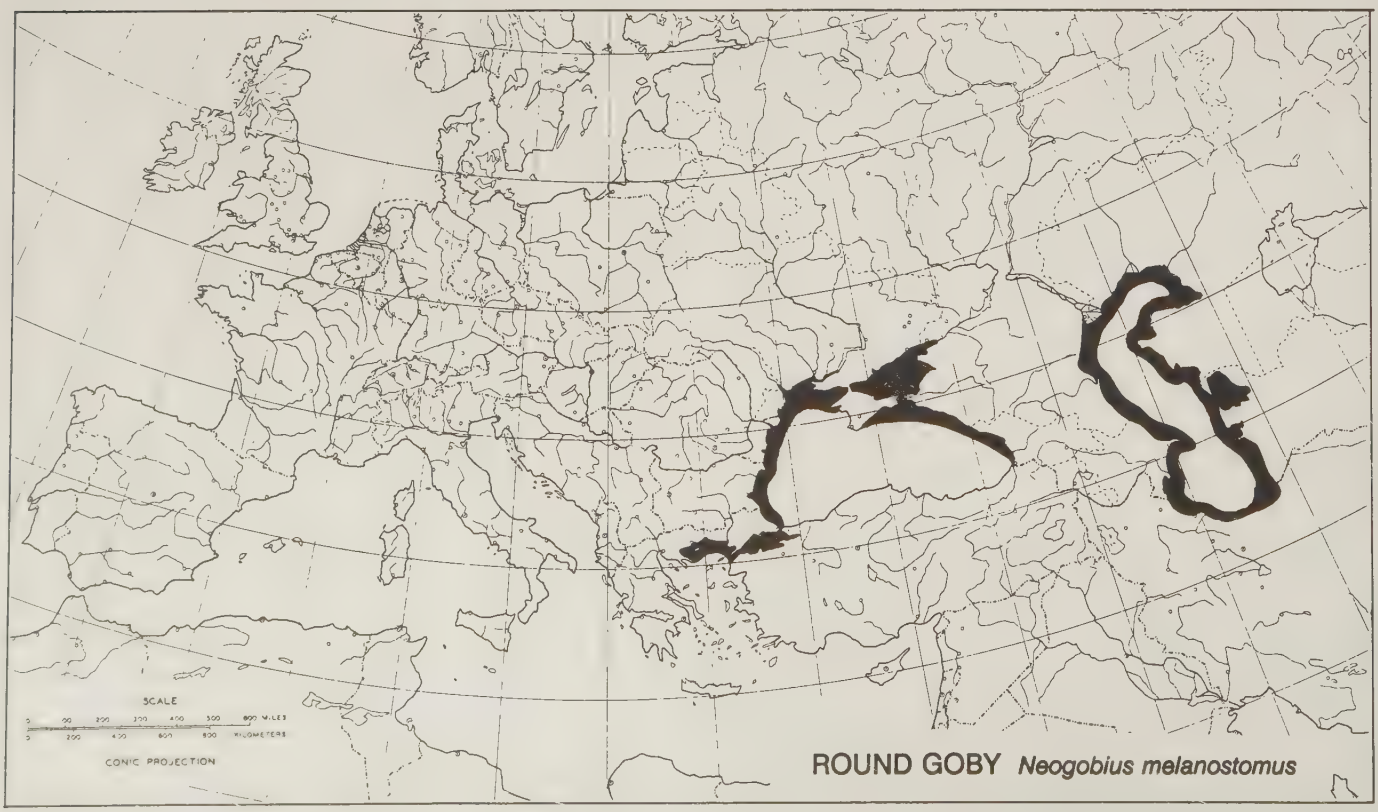

FIG. 5. Worldwide distribution map for the round goby (*Neogobius melanostomus*). Circles indicate major cities.

Cech 1988); these same two species were also transported via ballast water into Australia where they have become established and compete with native species (Hoese 1973). Another species is suspected of becoming established via oil supertanker ballast discharge in the Arabian Gulf (Al-Hassan and Miller 1987) and another was introduced into New South Wales (Middleton 1982). The ability of gobies to colonize new habitats is also exemplified by the rapid spread into the Aral Sea of several species of the genus *Neogobius* (Miller 1984b). However, as sampling in the St. Clair River has been very limited the last several years, it is difficult to identify when these fish may have actually been introduced into this waterbody. It appears based on the relatively high frequency of occurrence of tubenose gobies in our limited sampling efforts and the collection of at least two age groups of round gobies that these species may have successfully colonized the St. Clair River and will probably spread into the other Great Lakes with time. Their impact on native benthic fish communities could be severe.

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Bythotrephes cederstroemi (Schoedler) in Muskoka Lakes: First Records of the European Invader in Inland Lakes in Canada

Norman D. Yan

Ontario Ministry of the Environment, Water Resources Branch, Dorset Research Centre, P.O. Box 39, Dorset, Ont. P0A 1E0, Canada

Warren I. Dunlop

Ontario Ministry of Natural Resources, Muskoka Lakes Fisheries Assessment Unit, P.O. Box 1138, Bracebridge, Ont. P1L 1V3, Canada

Trevor W. Pawson

Ontario Ministry of the Environment, Water Resources Branch, Dorset Research Centre, P.O. Box 39, Dorset, Ont. P0A 1E0, Canada

and Lori E. MacKay

Ontario Ministry of Natural Resources, Muskoka Lakes Fisheries Assessment Unit, P.O. Box 1138, Bracebridge, Ont. P1L 1V3, Canada

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The large predaceous European zooplankter *Bythotrephes cederstroemi* is now present in eight large recreational lakes in south-central Ontario, Canada: Lake Muskoka, Lake Joseph, Lake Rosseau, Fairy Lake, Mary Lake, Peninsula Lake, Lake Vernon, and Go Home Lake. These observations represent the first evidence that *B. cederstroemi* has invaded inland lakes in Canada, and we suggest that close monitoring is now needed to establish whether or not pelagic food webs of the lakes will respond.

Bythotrephes cederstroemi, un prédateur zooplanctonique de grande taille originaire des eaux européennes, est maintenant retrouvé dans les eaux canadiennes. Il a été décelé dans huit grands lacs intérieurs du secteur centre-sud de l'Ontario utilisés à des fins de recréation, soit les lacs Muskoka, Joseph, Rosseau, Fairy, Mary, Peninsula, Vernon et Go Home. Selon les auteurs, une surveillance étroite permettra de déterminer si les réseaux alimentaires de la zone pélagique lacustre réagiront à la présence de cette nouvelle espèce.

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Bythotrephes cederstroemi Schoedler (Cercopagidae, Onychopoda) is a large, predaceous, Palaearctic zooplankter that invaded each of the Laurentian Great Lakes during the 1980's (Bur et al. 1986; Lange and Cap 1986; Lehman 1987; Evans 1988; Cullis and Johnson 1988; Jin and Sprules 1990). In Europe, *Bythotrephes* inhabits lakes that range widely in size, depth, hardness, acidity, colour, nutrient status, and fish assemblages (Nilsson and Pejler 1973; Hobæk and Raddum 1980; Næsje et al. 1987). Therefore, it is safe to assume that the majority of inland lakes in temperate North America offer suitable habitat to the invader. There was no evidence that *B. cederstroemi* has colonized any inland lake in Canada, despite the availability of habitat. It is our purpose to report the presence of *B. cederstroemi* in eight of the 12 largest lakes in the Moon River/Go Home River watershed in the District of Muskoka in central Ontario, Canada.

Study Lakes

The 12 study lakes (Table 1; Fig. 1) are located in the Moon River/Go Home River watershed, tertiary watershed No. 2EB of Cox (1978). The lakes are all large and have many cottages on their shores (Table 1). They have soft waters (range in conductivity 30–69 μS at 20°C) and are circumneutral in reaction, suffering little from the impacts of acid precipitation (pH 6.7–7.4, Gran alkalinity 60–220 $\mu eq \cdot L^{-1}$). They are oligotrophic, with total phosphorus levels generally <10 $mg \cdot m^{-3}$ and nitrogen/phosphorus ratios >30 by weight. Reid and Girard (1988) and Yan et al. (1991) provide additional information on the morphometry, water chemistry, and physical limnology of the lakes.

With the exception of Three Mile and Go Home lakes, the study lakes support fish communities dominated by lake trout (*Salvelinus namaycush*), smallmouth bass (*Micropterus dolomieui*), and coregonids. The fish communities of Three Mile and Go Home lakes are typical of more mesotrophic lakes, containing walleye (*Stizostedion vitreum*) and smallmouth bass. All 12 lakes also support yellow perch (*Perca flavescens*), as well as varying numbers of other percids, centrarchids, and cyprinids (Ontario Ministry of Natural Resources, unpubl. data).

Methods

Two sampling methods were employed routinely, one on Lakes Joseph, Muskoka, and Rosseau and a second on the remaining lakes. Zooplankton were collected from several widely spaced stations in Lakes Joseph, Muskoka, and Rosseau

on a monthly schedule over the ice-free seasons of 1989 and 1990 (Table 1). At each station, triplicate vertical hauls from 0.5 m above the sediments to the lake surface were taken with either an 11.5- or a 13-cm-diameter, nonmetered conical tow net constructed of 76-μm mesh. The contents of the three hauls were pooled prior to enumeration. Volumes of lake water sampled were calculated assuming 100% filtration efficiency, a reasonable assumption given the 13:1 filtering area to mouth area ratio of the net.

Zooplankton were collected from the remaining lakes in vertical hauls with a 46-cm-diameter, metered conical tow net constructed of 150-μm mesh. Hauls were taken through 30 m at stations deeper than 30 m and from 2 m above the bottom at shallower stations. Occasionally, zooplankton were also collected in horizontal hauls using either the 46-cm-diameter net, or a 47.5-cm-diameter, metered closing tow net constructed of 500-μm mesh. Samples were collected at several widely spaced stations in each lake (Table 1) in July or August of 1990 and/or in June of 1991. Measured filtration efficiencies, used to calculate volumes of lake water sampled, averaged 59.8%. Yan et al. (1991) provide maps showing all sampling locations.

B. Hutchinson (Ontario Ministry of the Environment, Dorset, Ont.) provided us with zooplankton samples she had collected as metered vertical net hauls from 14 stations in Lake Rosseau on July 27, 1983, 15 stations from Lake Muskoka on July 31, 1984, and 12 stations in Lake Joseph on August 8, 1984. To our knowledge, no historical zooplankton samples exist for the other study lakes.

All samples were preserved in the field with a 4–5% sucrose formalin solution. They were subsequently examined in their entirety for *B. cederstroemi*.

Results

Bythotrephes cederstroemi was not detected in any of the samples collected by Hutchinson from Lakes Muskoka, Rosseau, and Joseph in 1983 and 1984, nor was it reported by Michalski et al. (1973) in their 1969 collections from the lakes. In 1989 and 1990, *B. cederstroemi* was present in all three lakes. In the 1989 collections from Lake Muskoka, it was recorded in almost one third of the 45 collections and in four of the five months of sampling. In the 1990 collections from Lake Rosseau, it was recorded in all of the monthly sampling runs (Table 1).

Bythotrephes cederstroemi was recorded in the 1990 collections from Fairy and Mary lakes, but not in the six remaining lakes sampled in that year. Its absence from Lake Vernon and Peninsula Lake was a bit surprising because these lakes are connected with Fairy Lake by broad channels (Fig. 1) and are at the same elevation (Table 1). In the spring of 1991, *B. cederstroemi* was collected in horizontal hauls at the lake surface in both Lake Vernon and Peninsula Lake. Two animals were also collected at the last of five stations visited in Go Home Lake, downstream of Lake Muskoka (Table 1; Fig. 1).

In summary, the presence of *B. cederstroemi* is confirmed in eight of the 12 large lakes sampled in the watershed. Either

TABLE 1. Summary of selected data on the location and morphometry of the study lakes, on the sampling protocol for *B. cederstroemi*, and on the abundance of *B. cederstroemi* in the study lakes. The latitude (Lat), longitude (Long.), lake area, and elevation (Elev.) are provided, followed by the year of sampling (Year), the number of stations visited in the year (NS), and the number of dates (ND) all stations were visited. The gear code (GC)[a] and sampling protocol code (SC)[a] are indicated. *Bythotrephes cederstroemi* data summarized include the average detection limit (DL) for all stations and dates calculated assuming that one *B. cederstroemi* was observed in each sample. The percent of samples in which *B. cederstroemi* was observed is listed (Occ.), as is the total number of animals observed (Total no.) in the year. Finally the range in abundances of samples with *B. cederstroemi* is given along with the months in which it was observed (1, January; 2 February, etc.).

											Bythotrephes data				
Lake	Lat.	Long.	Area (ha)	Elev. (m)	Year	NS	ND	GC	SC	Mean DL (no.·m^{-3})	Occ. (%)	Total no.	Range in abundance (no.·m^{-3})	Month	No. of cottages
Fairy	4520	7911	711	284	1990	9	1	2	2	0.88	56	19	1.02–7.8	8	136
					1991	3	1	2	2	0.72	67	64	4.39–25.1	6	
Go Home	4501	7953	666	180	1991	5	1	2	2	0.56	20	2	3.05	6	433
Joseph	4511	7947	5156	225	1989	7	7	1	1	1.89	6	2	0.85–2.86	6, 7	792
					1990	9	6	1	1	1.36	5	3	0.35–0.73	7, 9	
Kawagama	4518	7845	2819	355	1990	7	1	2	2	0.89	0				900
Lake of Bays	4515	7904	6904	316	1990	12	1	2	2	0.72	0				1784
Mary	4515	7915	1065	281	1990	7	1	2	2	1.59	67	11	0.89–5.5	7	248
Muskoka	4500	7925	12200	225	1989	9	5	1	1	2.68	29	33	1.8–11.8	6, 7, 8, 10	3986
					1990	11	5	1	1	1.74	14	8	0.48–1.9	6, 7	
Peninsula	4520	7907	865	284	1990	2	1	2	2	0.61	0				313
					1991	2	1	2	2	0.51	0				
					1991	1	1	3	3		100	11	2.33	6	
Rosseau	4510	7935	6374	225	1989	7	5	1	1	3.26	14	8	1.17–6.5	7, 9, 10	907
					1990	10	6	1	1	2.05	18	11	0.29–17.5	6, 7, 8, 9, 10	
Skeleton	4515	7927	2154	281	1990	3	1	2	2	0.9	0				348
Three Mile	4510	7927	929	247	1990	4	1	2	2	8.6	0				340
Vernon	4520	7917	1505	284	1990	2	1	2	2	0.66	0				337
					1991	1	1	2	2	0.35	0				
					1991	1	1	3	3		100	6	0.32	6	

[a]Gear codes: 1, 76-μm mesh, 11.5- or 13-cm diameter, nonmetered conical tow net; 2, 150-μm mesh, 46-cm diameter, metered conical tow net; 3, 500-μm mesh, 47.5-cm diameter, metered closing conical tow net. Sampling codes: 1, pooled triplicate vertical hauls from 0.5 m above bottom; 2, single vertical haul from 30 m, or from 2 m above bottom if the station was <30 m deep; 3, horizontal haul at the lake surface.

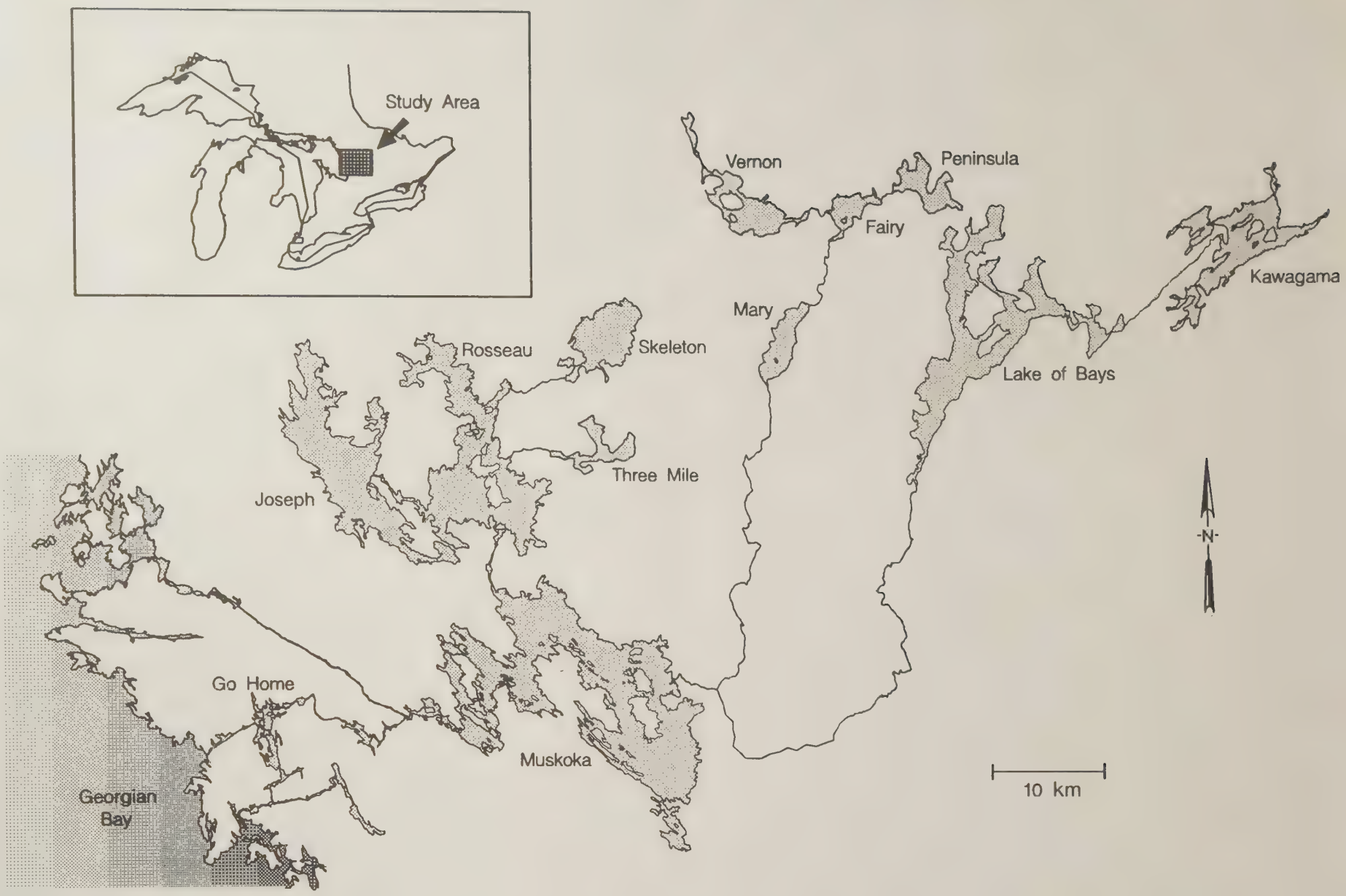

FIG. 1. Location of study lakes in the watershed.

the invader was not present in the remaining four lakes or it was present at densities below our limits of detection. These limits can be approximated from the summed sample volumes to be 0.2, 0.67, 0.053, or 0.08 animals·m^{-3} for Skeleton, Three Mile, Lake of Bays, and Kawagama lakes, respectively.

Discussion

Bythotrephes cederstroemi has been observed in only one other set of inland lakes in North America. Jeff Schuldt and Glen Merrick (University of Minnesota at Duluth. pers. comm.) collected *B. cederstroemi* in plankton hauls in Fish, Boulder, and Island lakes, three lakes in the Cloquette River watershed in northern Minnesota, USA, in September 1990. They suspected that *B. cederstroemi* had been introduced into these lakes by anglers.

Anglers may also have introduced *B. cederstroemi* into the Muskoka lakes. The lakes support important summer and winter recreational fisheries for lake trout, smallmouth bass, and walleye, for example, 20 000 to 40 000 angler-hours·yr of $effort^{-1}$ on Lakes Rosseau and Joseph (Ontario Ministry of Natural Resources, unpubl. data). We asked operators of marinas on Lake of Bays, Lakes Muskoka, Rosseau, and Joseph, and Fairy Lake if boats are moved from the Great Lakes into their lakes. They confirmed that such movement does occasionally occur. *Bythotrephes cederstroemi* could have been introduced into the inland lakes by anglers or other boaters in various ways, the most obvious being spillage of Great Lakes water containing *B. cederstroemi* from livewells of fishing boats or from anglers' bait buckets. Anglers are also suspected of accidentally or intentionally introducing northern pike (*Esox lucius*), margined madtom (*Notorus insignis*), and black crappie (*Pomoxis nigromaculatus*) into various lakes in the watershed.

The marina operators also indicated that boats are frequently moved among our study lakes. This should speed the dispersal of *B. cederstroemi* within the watershed, now that it is established. There are 2022 lakes >1 ha in this watershed (Cox 1978).

Of course, we cannot be certain how *B. cederstroemi* invaded the Muskoka lakes. The lakes are all isolated from Georgian Bay by several waterfalls and rapids; hence, colonizers could not have entered the lakes directly. In addition to recreational boaters or anglers, waterfowl may have served as the vector of colonization. It would be a short flight for migrating waterfowl from Georgian Bay to any of the colonized lakes (Fig. 1). Jin and Sprules (1990) did report *B. cederstroemi* at all seven stations they visited in Georgian Bay in 1988.

At the moment we do not know how, or even if, the pelagic food webs of the Muskoka lakes will respond to the presence of *B. cederstroemi*. The European literature provides little guidance because the consumption rates of *B. cederstroemi* have not been compared with the production rates of its prey in any European lake. The impacts of the invader on the plankton assemblages of the Laurentian Great Lakes are also a source of controversy. Lehman (1988) and Scavia et al. (1988) suggested that *B. cederstroemi* has reduced the densities of *Daphnia* in Lake Michigan; however, Sprules et al. (1990) reported no evidence of such a decline in their Lake Michigan data. Because investigations such as these are in their early stages, and because Great Lakes food webs may not yet have stabilized in response to the invader, the full impacts of *B. cederstroemi* on the Great Lakes may not be understood for some time.

The impacts of *B. cederstroemi* on Shield lakes will not be understood without research. Obvious initiatives which would improve our understanding include studies of (1) the abundance, spatial distribution, migratory behaviour, production, and life history of *B. cederstroemi* populations in several Shield lakes with differing fish assemblages (*Bythotrephes* is less abundant in European lakes with large planktivorous fish populations (De Bernardi and Giussani 1975)), (2) the interaction of *B. cederstroemi* with other large, planktonic macroinvertebrate predators in Shield lakes, including mysids, mites, larval *Chaoborus*, and *Leptodora*, (3) prey preferences of *B. cederstroemi* in Shield lakes, (4) predation rates of *B. cederstroemi* and production rates of their preferred prey, and (5) the spread of *B. cederstroemi* across the Shield. Little or no historical zooplankton data exist for the vast majority of Shield lakes that will ultimately be colonized by *B. cederstroemi*. Therefore, paleolimnological assessments of changes in zooplankton assemblages may also be a fruitful avenue of research (e.g. Kitchell and Kitchell 1980).

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CORRECTIONS TO RECENT PUBLICATIONS
CORRECTIONS SUR RÉCENTES PUBLICATIONS

VOLUME 48, No. 10 — Article by M. Hanna and R. H. Peters: *Effect of sampling protocol on estimates of phosphorus and chlorophyll concentrations in lakes of low to moderate trophic status.*
Pages 1985 and 1986: The following six references contained errors and should read as follows:

BERGMANN, M., AND R. H. PETERS. 1980. A simple reflectance method for the measurement of particulate pigment in lake water and its application to phosphorus–chlorophyll–seston relationships. Can. J. Fish. Aquat. Sci. 37: 111–114.

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